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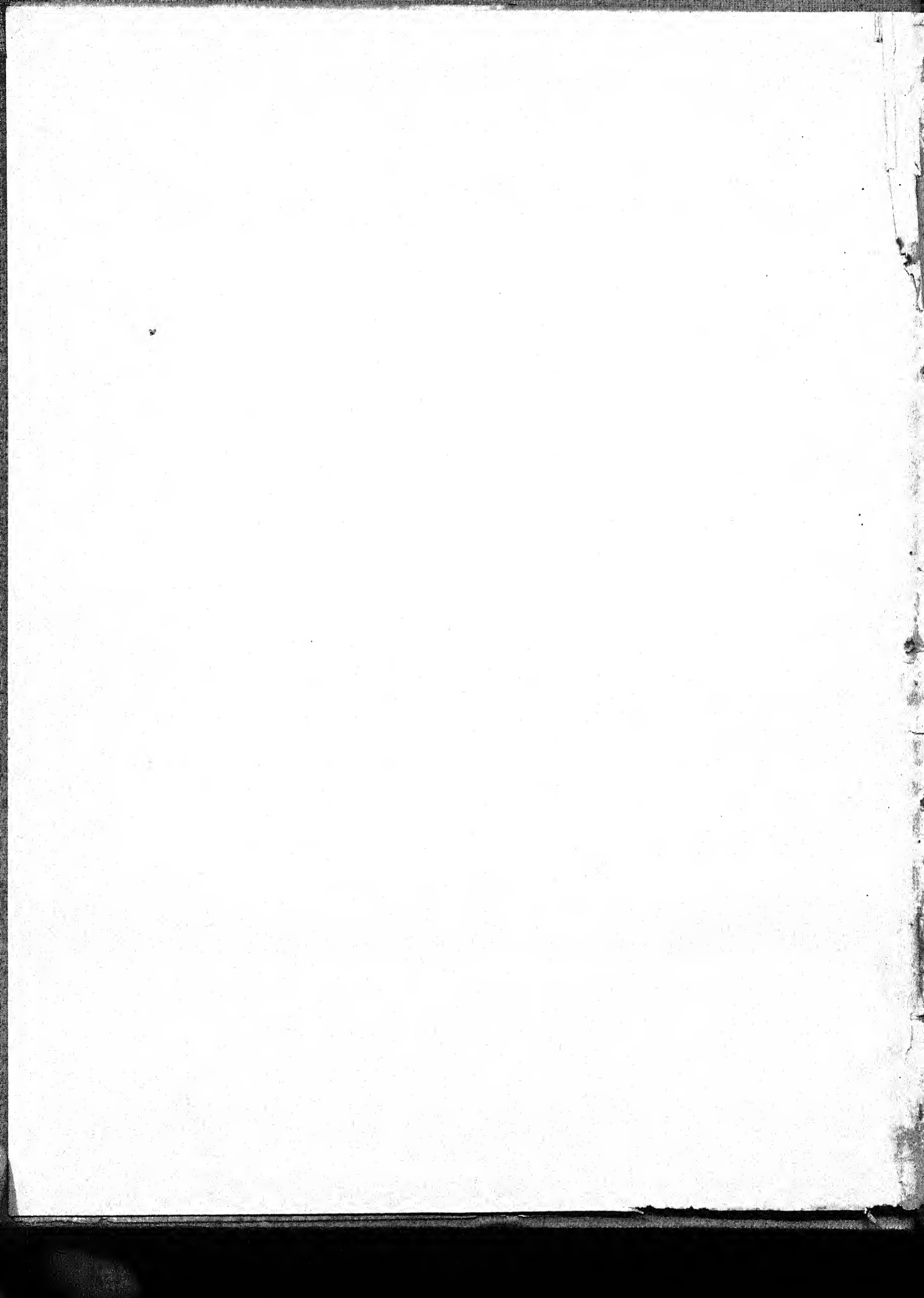
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# INVESTIGATIONS ON INDIAN OPIUM.

## NO. 1. NON-ENVIRONMENTAL FACTORS INFLUENCING THE ALKALOIDAL CONTENT AND YIELD OF LATEX FROM THE OPIUM POPPY (*PAPAYER SOMNIFERUM*).

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### PART I.

#### INTRODUCTION AND ECONOMIC ASPECT.

THE work described below represents an investigation arising out of war conditions. In pre-war days the bulk of medicinal opium was provided by Asia Minor, the Balkans and Persia.

The following table shows<sup>1</sup> the quantities and value of the imports of opium into the United Kingdom with their countries of origin during the years 1913 and 1914.

<sup>1</sup> *Bull. Impl. Inst.*, Vol. XIII, 1915, p. 508.

TABLE I.

From				1913		1914	
				Cwt.	£	Cwt.	£
Hong Kong	..	..	..	339	38,102	267½	26,426
Persia	..	..	..	1,219	142,852	2,330	247,464
Asiatic Turkey	..	..	..	1,940	174,878	2,690	242,045
European Turkey	..	..	..	1,387	133,858	1,362	126,588
Other countries	..	..	..	176½	17,572	1,030½	94,159
GRAND TOTAL				5,061½	507,262	7,680	736,682

The next table gives figures for exports of opium from Turkey during the years 1909 to 1913. Those for 1909-10 are taken from a report from Prof. W. R. Dunstan, F.R.S., to the India Office in 1913. Those for 1911-13 were supplied by H. A. F. Lindsay, Esq., I.C.S., Director-General of Commercial Intelligence, and are incomplete.<sup>1</sup>

TABLE II.

From	1909		1910		1911		1912		1913	
	Cwt.	Value £	Cwt.	Value	Cwt.	Value	Cwt.	Value	Cwt.	Value
Salonica ..	1,247*	87,300	2,357*	165,000	1,640	..	2,640	..	..	..
Smyrna ..	3,800	..	4,800	..	3,800	..	2,980	..	..	..
Basra ..	1,841	..	1,791	..	1,570	117,500	1,307	97,600	1,840	13,750
Trebizond	1,280	102,400	1,321*	105,680	..	..	..	101,520	..	..

\* Quantities recorded in the returns. The figures here given are calculated from the values and are only approximate.

The total exports of opium from Turkey<sup>2</sup> during the year March 1911 to February 1912 were 306 tons, value £692,000, of which 183 tons, value £412,000, came to the United Kingdom.

According to the *Encyclopædia Britannica*,<sup>3</sup> the average amount of Turkish opium exported in pre-war days was 7,000 chests (about 470 tons) but in rare seasons amounted to 12,000 chests (about 800 tons).

1 "Report on the Ghazipur Opium Factory," by W. A. Davis, 1918.

2 *Bull. Imp. Inst.*, Vol. XIII, 1915, p. 508.

3 *Enc. Brit.*, 11th Ed., Art. on Opium, p. 132.

Wiesner<sup>1</sup> gives the average annual production in Asia Minor alone as 400,000 kg. (357 tons).

The production of Persian opium cannot be given with any degree of certainty and much of it is exported to Eastern Markets. The amount of it used for medicinal purposes is small in comparison with that from Turkey. From a study of the foregoing figures, we may put the average out-turn of medical opium per annum from Turkey and Persia in pre-war days at about 500 tons. Opium was subject to great variations in price, this amounting to from 10s. to 26s. per lb. The lower price would put the pre-war value of the medical opium trade at £1,120,000 and the higher at £2,912,000.

After the outbreak of war with Turkey the Turkish opium supplies to the Allies ceased. The demand for opium and its products on the other hand increased. The possibility of the use of Indian opium for medicinal purposes was then brought into prominence. The *British Pharmacopœia* (1898) lays down as the standard for medical opium that when dry it should yield not less than  $9\frac{1}{2}$  per cent and not more than  $10\frac{1}{2}$  per cent of anhydrous morphine. For the preparation of tinctures, opium containing not less than 7.5 per cent morphine may be used, and opium containing more than  $10\frac{1}{2}$  per cent morphine may be reduced to the medical standard by dilution with *milk sugar or with opium containing not less than 7.5 per cent morphine.*

We might refer here to the standards laid down in other countries for medical opium.

*United States.* In its normal moist condition opium must yield not less than 9 per cent of crystalline morphine. For opium granulatum, *i.e.*, opium dried at a temperature not exceeding 85° C. and reduced to a coarse powder, the standard is 12— $12\frac{1}{2}$  per cent of crystalline morphine.

*France.* When dried at 60 °C. opium must contain 10—11 per cent of morphine.

*Germany.* Opium must contain 10—12% morphine and not more than 8 % of water.

*Japan.* Opium must contain 10—11% morphine in the dry state.

It is important to observe that the methods of morphine assay in opium vary in different countries, and different methods of assay give results which vary at least 1 per cent among themselves.

<sup>1</sup> Wiesner, *Die Rohstoffe des Pflanzenreiches*



Turkish opium easily reaches the medicinal standard. The following analyses<sup>1</sup> show the composition of typical Turkish and Persian opiums as they come on the market.

Moisture		Mineral matter	Aqueous extract	Matter insoluble in water	Anhydrous morphine	Anhydrous morphine in dry opium
%		%	%	%	%	%
<i>Turkey opium.</i>						
16.4	..	5.1	49.9	35.7	11.15	13.34
20.2	..	3.9	50.2	29.6	8.56	10.73
18.0	..	3.6	53.6	28.4	9.20	11.22
16.9	..	4.4	46.2	36.9	12.31	14.81
17.8	..	4.8	49.9	32.3	10.97	13.35
21.5	..	4.3	55.0	23.5	9.95	12.68
<i>Persian opium.</i>						
10.5	..	4.8	59.4	30.1	11.25	12.57
14.8	..	3.2	57.9	27.3	9.40	11.04
28.2	..	3.1	55.0	16.8	8.00	11.14
17.6	..	4.6	57.8	24.6	10.35	12.41
18.1	..	4.8	55.9	26.0	10.80	13.18
14.5	..	5.1	60.0	25.5	11.65	13.63

Indian opium was stated to contain only 6 to 8 per cent of morphine in the dry substance and was therefore unable to compete with the Turkish for the medical trade. The Indian Government have for many years past had under consideration the capture of this trade.

The files of the *Pharmaceutical Journal*<sup>2</sup> in the last decade of the last century make occasional suggestions that India might seriously consider the capture of the medical opium trade. In 1896 the Imperial Institute made analyses of Indian opium<sup>3</sup> and preliminary trials with it at St. Thomas' Hospital being favourable, the Institute authorities drew the attention of the Government of India to the possibilities of employing Indian opium in European medicine. The Government of India were, however, then of opinion that any attempt to introduce Indian opium into Europe might prejudice the existing trade with China.<sup>4</sup>

In 1907 the question was again reviewed owing to the opium treaty with China by which the exports of opium from India to China were to be annually diminished until the trade ceased. Samples of Indian opium were sent to the Imperial Institute for examination. A report based on these analyses

<sup>1</sup> Parry, "Food & Drugs," Vol. I, p. 583.

<sup>2</sup> *Pharm. Jour.*, 1892, p. 505; 1895, p. 207.

<sup>3</sup> *Bull. Imp. Inst.*, *loc. cit.*

<sup>4</sup> Lord Elgin's despatch No. 46 of 1897 from the Govt. of India, through *Bull. Imp. Inst.*, *loc. cit.*

was submitted to the Government and finally printed in the Bulletin of the Imperial Institute.<sup>1</sup>

From time to time certain suggestions have been thrown out to explain the reason for the low morphine content of Indian opium and we propose to mention the chief of them here. The Indian climate has been blamed, as will be seen later in the description of our work. It has also been ascribed to lack of soil aeration. Thus Howard<sup>2</sup> writes:—"This (*i.e.*, Indian) opium is too poor in morphine and falls below the morphine standard required by the British Pharmacopœia. The problem is to increase the quality. I venture to suggest that this will also be found to be an aeration effect and to result from an insufficient supply of air in the soil. If the opium land is drained on the Pusa system and if *thikra* (at the rate of about 30 to 50 tons per acre) is mixed with the upper six inches of soil I believe the quality of the Oudh opium will instantly improve." Against this scheme we would note that in 1916-17 there were over 300,000 acres under poppy in the United Provinces. Moreover, it is difficult to understand why Indian soils should be the only ones amongst poppy-growing countries which should be lacking in soil aeration.

The *Encyclopædia Britannica*<sup>3</sup> states that "In view of the probable decline in the Chinese demand, the cultivation of the drug for the European market in the hilly districts of India and its preparation after the mode adopted in Turkey, *viz.*, by drying the concrete juice as quickly as possible, might be worthy of the consideration of the British Government."

Wiesner<sup>4</sup> quoting from the *Pharmaceutical Journal* ascribes the low morphine content of Indian opium to faulty methods of harvesting and preparation.

Dunstan<sup>5</sup> points out the difference in methods of lancing in India and Asia Minor and suggests that the Asia Minor methods be used in India. His suggestion apparently was made with the idea of saving labour in India, however, and not with the idea of improving the morphine content of the Indian commodity.

Thorpe<sup>6</sup> ascribes the low morphine content of the bulk of Egyptian opium as due partly to an overmoist soil and unskilful collection.

1 *Bull. Imp. Inst.*, 1915, Vol. XIII, p. 507.

2 *Bull. Agr. Res. Inst., Pusa*, 61, 1916, p. 21.

3 *Art. on Opium*, 11th Edn., p. 134.

4 Wiesner, *Die Rohstoffe des Pflanzenreiches*, 1900, I, p. 410.

5 *Bull. Imp. Inst.*, 1915, Vol. XIII, p. 346.

6 *Dict. of Applied Chemistry*, Vol. IV, p. 16.

With the continuance of the war Government decided to have work done in India to try to discover the reason for the low morphine content of Indian opium. The problem seemed a chemical one, and in October 1916 the senior author was placed on special duty to study the subject. The reason for the inferiority of Indian opium in morphine content was fortunately discovered in the first season's work. The remedy is a simple one and India should now produce opium with as high a morphine content as that from Turkey. A number of subsidiary factors have, however, been discovered which influence morphine content in opium.

The influence of various factors will be studied in this series of papers. The opportunity has also been taken of summarizing work done by other workers on the factors influencing amounts of active principles produced by other plants. We consider our results also throw considerable light on the question of the functions of alkaloids in plants.

## PART II.

### DESCRIPTION OF THE METHOD OF EXTRACTION OF THE LATEX.

It will make the description of our work easier if we first describe the method of extraction of the opium as practised by the Indian cultivators. The operation is commenced some 10 to 20 days after the fall of the flower petals. The capsules are considered to be ready for lancing as soon as they feel firm to the touch and are yet green. The instruments used are :—

(1) A knife which consists of 3 or 4 parallel sharp-pointed blades bound together with cotton, the binding being so done that the points of the blades are about  $\frac{1}{8}$ " apart and all in the same plane.

(2) A small iron scoop.

(3) Unglazed earthen pots in which to store the opium.

The cultivator then usually divides his field into three portions, A, B, C. The lancing is invariably commenced just after midday and is carried out by men, women and children. They begin at the edge of the field and work backwards, as otherwise the exuding latex would be brushed off on their clothes. The hand is quickly passed over the capsules. If a capsule is considered to be ready it is grasped in one hand and the knife is drawn vertically upwards over its surface from just above the stalk to just below the stigmatic rays at the top of the capsule. Sometimes the cut is made downwards. The second finger of the hand holding the knife is placed near the points of the knife on the surface of the capsule to steady the motion of the knife. Great care is taken not to cut too deeply into the capsule. If the

cut be made too deep then the wall of the capsule may be cut completely through with consequent secretion of the latex on the inner surface. Good operators can incise 150 to 200 capsules in an hour.

Immediately on incision the latex commences to flow. It varies in colour from milk white and smoky white through pale pink to a very bright pink. It rapidly begins to darken, however. Next morning at 6 a.m., or later if there is a heavy dew, the opium is collected. It is scraped off the cut surface with the blunt-edged iron scoop. From the scoop it is transferred to earthen pots. Usually the cultivator lances only one-third of his field (*e.g.*, portion A) on the first day. After collecting the opium next morning from that portion he then lances portion B, that is on the second day. On the third day the portion C is lanced. On the fourth day he again returns to lance portion A. On this occasion many more heads are now ready for a first lancing. In addition a second lancing is given to those capsules which were lanced on the first day. On the fifth day portion B receives its second lancing, and on the sixth day portion C receives its second lancing. On the seventh day portion A is lanced for the third time and on this occasion more heads are usually ready for a first lancing. Those lanced already once, now receive a second lancing. Portions B and C come in for similar treatment in regular rotation. The lancements are continued as long as the capsules continue to give any appreciable yield of latex. Usually each field is lanced four or five times and occasionally as many as eight times, so that some heads might then have received eight lancements. In some poor seasons and in some poor crops the heads cease to yield opium after the second lancing. The whole opium produce of each field is mixed together and after three to six weeks' storage is brought to an official of the Government Opium Department by whom each cultivator's opium is weighed and examined for consistency or dry matter by touch, and for certain adulterations (*e.g.*, starch by iodine test). The opium officers reach such a high degree of skill that they can usually tell the moisture content of the opium by touch to within 2 or 3 per cent. The cultivator at once receives the value of his opium except for a small balance which is paid some two months later when the district officers' examination has been checked by analysis at the Government Opium Factory, to which place all the opium is sent.

### PART III.

#### THE ALKALOIDAL CONTENT AND YIELD OF LATEX FROM EACH OF A SERIES OF SUCCESSIVE LANCINGS OF THE SAME CAPSULE.

We have established the fact that on the first occasion on which a capsule is incised by the cultivator the opium is far richer in morphine than is the

produce of the second lancing and this again than the third lancing and so on. Cases have been met with in which the opium obtained from the fifth lancing gave no morphine at all by the method of estimation laid down in the *British Pharmacopœia*. The literature makes no reference to analyses of opium from a series of successive lancements of the same capsules. After we had made our discovery we found the following statement in an ably written note by Mr. Levett-Yeats<sup>1</sup>: "The variations in the produce of the same variety of plant may be due to difference of soil, climate and cultivation or to successive scarifications. The drug of the later scarifications may be weaker in morphine and so reduce the morphine content." Mr. Levett-Yeats has since informed us that he had no facilities to follow up the matter. We have made a very large number of analyses of opium of the first and subsequent lancements and most of these figures are set out in the accompanying tables. In most cases the actual yields of opium obtained at each lancing are also recorded.

In Table I are set out analyses of the opium produced from the first, second, third and subsequent lancements of each plot. At the second lancing some fresh heads will be ready for their first lancing and hence the opium of the second lancing includes opium from heads which have been lanced for the second time, as well as opium from heads which have been lanced for the first time. Similarly, at the third lancing more capsules will be ready for a first lancing and the opium collection will include the produce of heads lanced thrice, twice, and for the first time and so on.

In Table II are set out analyses of opium of the successive lancements of the same heads, no new heads being included at the second or subsequent lancements. In the Tables I and II are inserted figures to show the yield of opium at each lancing. In Table II yields are expressed as grm. of dry opium per 1,000 capsules.

In a number of experiments we have modified the usual method of lancing by making three cuts with the knife on each head instead of one.

Each table is therefore subdivided into two portions, the first showing results obtained by the ordinary method of lancing and the second showing results obtained by the modified method of treble incisions at each lancing. It will be seen that the yields of the second and subsequent lancements rapidly diminish in the case of the new method of lancing.

<sup>1</sup> Note to Government by Mr. Levett-Yeats, Factory Superintendent, Ghazipur Opium Factory, dated 6th December, 1913, on Prof. Dunstan's Report on Indian Opium.



TABLE I.

Showing yield of opium per plot at each successive lancing and morphine content of the same.

Description of plot	GRM. OF OPIUM DRIED AT 100°C. PER PLOT FROM EACH SUCCESSIVE LANCING								MORPHINE CONTENT OF DITTO						
	1st	2nd	3rd	4th	5th	6th	7th	Total	1st	2nd	3rd	4th	5th	6th	7th
(a) Ordinary country method of lancing 1916-17															
Cawnpore Field 29 Plot ..	1	82.0	91.3	31.4	15.0	4.7	0.9	..	225.3	10.9	7.7	3.6	3.5	2.7	..
	2	75.5	75.1	55.4	17.6	7.8	2.0	..	233.4	10.4	8.8	3.9	2.9	2.6	..
	3	42.5	65.1	49.8	16.5	3.8	1.5	..	179.2	9.0	9.1	5.0	4.1	..	..
	4	29.4	27.5	42.5	13.3	6.1	2.9	..	121.7	11.5	—	4.9	3.9	3.1	..
	5	19.0	67.1	52.3	25.8	8.1	2.0	..	174.3	9.3	8.0	4.3	6.0	2.7	..
	6	37.3	34.6	38.6	20.3	6.6	4.3	..	141.7	9.7	7.2	5.8	3.8	2.6	2.3
	7	27.5	29.6	40.5	15.3	4.2	2.0	..	119.1	9.6	8.6	5.8	4.0	2.3	..
	8	15.2	35.9	36.3	24.7	5.3	3.6	..	121.0	8.1	8.0	3.7	4.1	2.3	..
	9	67.1	63.4	37.8	15.2	4.5	2.3	..	190.3	9.3	4.9	4.3	4.0	1.5	..
	10	47.3	51.9	42.6	20.4	7.0	3.3	..	172.5	9.9	6.3	4.4	1.4	..	..
	11	79.4	70.0	61.7	29.9	8.7	2.7	..	252.4	10.4	7.6	5.2	4.8	1.9	..
	12	52.2	69.5	52.3	28.8	5.8	1.1	..	209.7	10.0	7.6	4.4	3.3	2.0	..
	13	46.5	43.7	38.0	15.7	4.0	3.4	..	151.3	10.8	7.9	5.3	3.0	1.3	..
	14	46.4	55.9	40.0	13.3	2.5	0.8	..	158.9	10.7	8.1	3.5	3.3	..	..
	15	83.3	66.6	47.7	13.1	4.0	2.5	..	217.2	10.9	6.9	3.4	3.5	..	..
	16	51.1	47.9	35.1	14.6	2.7	0.2	..	151.6	10.6	7.3	3.8	3.7	..	..
Cawnpore Field 9 Plot ..	1	24.3	54.4	42.8	14.5	9.9	5.5	2.2	153.6	11.8	9.8	6.5	..	3.4	1.8
	2	41.3	61.9	34.9	12.3	7.8	4.7	1.9	164.8	10.9	7.8	5.4	..	1.6	1.4
	3	25.6	57.2	32.5	13.5	7.6	2.0	1.6	140.0	10.7	8.8	4.8	..	2.0	..
	4	19.6	37.1	30.0	10.8	4.7	2.0	1.0	105.2	10.4	8.7	4.3	..	2.0	..
	5	20.2	38.1	38.6	24.8	9.5	5.5	2.2	138.9	11.7	8.8	6.4	4.8	3.3	2.0
	6	25.6	35.0	32.7	17.0	10.9	4.6	3.5	129.3	11.2	9.1	3.8	4.0	2.5	1.2
	7	8.9	29.0	33.4	19.0	8.4	4.6	3.5	106.8	10.3	8.7	5.6	5.3	2.3	1.9
	8	16.1	27.4	31.2	13.3	8.7	5.4	2.4	104.5	9.6	8.0	5.5	..	2.5	1.6
	9	41.6	41.5	49.4	29.0	32.2	13.3	6.8	213.8	11.6	9.9	5.0	..	3.7	2.4
	10	20.5	30.0	34.2	30.3	16.0	7.6	5.2	143.8	11.1	9.6	7.1	..	3.9	1.3
	11	35.8	49.6	44.2	24.6	12.4	10.6	8.2	185.4	10.9	10.7	7.3	..	2.8	2.4
	12	27.1	44.2	49.0	30.5	13.6	8.9	3.5	176.8	11.6	10.0	7.9	..	3.7	2.0
	13	14.0	32.1	32.4	22.7	10.7	4.6	1.9	118.4	10.9	9.6	4.4	..	1.9	2.3
	14	18.5	32.3	23.2	14.0	5.7	1.5	0.4	95.6	11.1	8.3	5.0	..	1.3	..
	15	27.9	56.9	40.2	19.9	7.6	7.0	2.5	162.0	11.2	9.6	6.2	4.0	1.9	1.3
	16	15.7	40.8	32.7	18.5	6.5	4.3	1.6	120.1	10.7	8.8	6.0	..	1.4	1.2
Cawnpore variety Katela ..	..	594.9	804.2	593.6	468.2	185.1	97.1	..	2,743.1	11.2	9.3	6.4	3.4	2.0	1.3
Cawnpore Field 55 Plot ..	2	22.2	21.4	19.8	19.2	8.9	..	..	91.5	11.6	10.9	11.9	7.4	6.7	..
	3	18.5	32.7	17.6	10.7	5.3	..	..	84.8	12.6	10.6	8.9	4.2	4.3	..
	5	12.8	23.5	13.8	10.0	3.1	..	..	63.2	11.5	10.0	8.3	5.9	..	..
	7	9.0	18.9	27.7	31.9	11.1	..	..	98.6	14.3	11.5	11.5	8.5	6.7	..
	10	19.9	22.4	24.4	27.7	7.4	..	..	101.8	13.1	10.9	10.4	6.4	5.2	..
	12	19.2	23.3	11.9	11.3	4.0	..	..	69.7	13.1	10.5	7.6	5.0	4.8	..
	13	15.1	23.2	14.4	13.5	2.9	..	..	69.1	11.9	11.3	8.4	5.2	..	..
Cawnpore Cultivators—															
Chalmu ..	..	..	..	..	..	..	..	Yields not-recorded	..	9.5	4.9	3.6	1.8	..	..
Jwala ..	..	..	..	..	..	..	..	..	..	..	5.2	4.6	4.4	..	..
Mangli ..	..	..	..	..	..	..	..	..	9.2	5.6	..	..	..	..	..
Douglas Dale—															
Katela ..	1	..	..	..	..	..	..	..	10.5	6.8	3.8	3.1	..	..	..
Katela ..	2	..	..	..	..	..	..	..	10.3	8.2	..	..	..	..	..

TABLE I—(continued).

Description of plot	GRAM. OF OPIUM DRIED AT 100°C. PER PLOT FROM EACH SUCCESSIVE LANCING								MORPHINE CONTENT OF DITTO						
	1st	2nd	3rd	4th	5th	6th	7th	Total	1st	2nd	3rd	4th	5th	6th	7th
(a) Ordinary country method of lancing 1917-18--(continued).															
<i>Cawnpore</i> Field 44															
Plot .. 1	91.4	85.1	24.7	18.8	10.5	4.0	..	234.5	15.0	11.8	8.8	6.2	3.7	2.0	..
2	180.4	157.0	53.9	54.4	30.3	13.4	..	489.4	15.7	11.7	9.5	5.4	2.3	1.6	..
3	164.5	124.1	89.5	33.9	5.2	3.2	..	420.4	15.0	13.0	8.1	6.1	3.3	2.5	..
4	132.9	126.3	68.0	29.7	11.8	2.7	0.4	371.8	14.3	11.4	7.9	6.3	4.8	2.1	..
5	78.5	66.2	24.1	13.8	7.4	1.6	0.6	192.2	15.1	12.1	8.7	5.9	3.6	..	..
6	169.7	134.9	39.1	12.4	6.8	1.7	0.4	365.0	15.3	11.1	8.7	6.4	4.0	..	..
7	70.7	83.1	52.7	10.7	11.6	3.6	0.7	233.1	15.8	12.0	8.0	6.3	4.2	1.2	..
8	72.1	67.8	35.7	12.5	9.0	2.2	0.5	199.8	15.1	11.5	8.6	7.4	4.5	1.4	..
9	67.7	50.4	32.7	24.6	12.0	6.4	..	193.8	14.7	12.4	9.4	6.3	3.0	2.3	..
10	123.7	88.9	40.3	22.2	9.8	3.2	..	288.1	15.7	12.5	10.3	5.1	4.1	2.6	..
<i>Cawnpore</i> Field 75															
Plot .. 1	35.5	12.4	5.4	2.1	0.4	0.1	..	55.9	12.5	12.4	7.0	2.9	1.6	..	..
4	25.9	12.5	5.1	2.0	0.4	..	..	45.9	13.8	10.1	7.0	4.9	2.8	4.0	..
<i>Ankin</i> Plot III	1	235.2	324.7	258.0	180.4	32.0	..	1030.3	15.7	12.5	9.5	7.7	..	..	..
<i>Experiments on cul- tivators' fields.</i>		321.9	576.5	411.7	131.0	44.1	..	1485.2	16.7	13.7	10.3	8.5	7.1	..	..
<i>Ballia</i>															
Village Rampur A	1010.8	583.5	323.2	174.0	67.3	25.5	..	2183.5	11.6	8.3	5.9	..	..	..	..
C	659.7	321.5	189.1	91.2	35.9	..	..	1297.4	11.9	7.7	6.6	..	..	..	..
<i>Dalmau</i>															
Lala's Field .. 1	259.4	295.9	105.3	31.8	26.8	6.4	..	725.6	10.3	5.7	4.4	..	..	..	..
3	398.8	291.6	101.5	25.6	29.4	3.1	..	850.0	9.7	5.5	4.1	..	..	..	..
<i>Bhagwan's</i> Field .. 1	181.7	329.4	293.9	161.3	52.7	12.5	..	1031.5	12.4	10.6	8.6	..	..	..	..
2	158.4	237.9	271.4	177.5	68.4	6.3	..	919.9	11.2	11.7	9.6	..	..	..	..
<i>Rae Bareilly</i>															
A	307.8	376.8	199.7	131.5	70.8	..	..	1086.6	13.8	10.0	7.1	..	..	..	..
C	452.0	407.0	136.0	125.0	..	..	..	1120.0	12.8	9.4	7.7	..	..	..	..
<i>Fyzabad</i>															
Saktu Teli's Field 1A	322.9	392.0	129.3	..	..	..	..	844.2	13.1	9.4	5.5	..	..	..	..
2A	491.2	196.4	42.1	..	..	..	..	729.7	13.9	9.4	6.9	..	..	..	..
3A	318.2	184.8	31.3	..	..	..	..	534.3	13.6	8.5	6.5	..	..	..	..
<i>Patan Mur's</i> Field 1A	510.6	354.0	..	..	..	..	..	864.6	14.7	9.8	..	..	..	..	..
2A	646.8	282.0	..	..	..	..	..	928.8	13.7	7.5	..	..	..	..	..
3A	581.6	207.8	..	..	..	..	..	789.4	12.3	9.2	..	..	..	..	..
<i>Ghazipur</i>															
Plot .. A	400.1	193.9	189.0	88.4	..	..	..	871.4	11.3	8.1	9.2	..	..	..	..
D	384.2	172.7	200.0	52.2	..	..	..	809.1	11.9	9.7	8.1	..	..	..	..
X	21.8	35.0	67.3	50.5	21.4	12.9	..	208.9	12.8	13.2	13.0	11.4	10.8	..	..
Y	42.7	94.0	64.8	49.8	16.8	6.6	..	274.7	13.1	13.4	12.8	11.0	10.9	..	..
Z	19.4	48.7	56.3	25.9	..	5.4	..	155.7	13.4	13.6	12.3	11.0	..	..	..
<i>Lucknow</i>															
.. A	225.5	166.3	..	..	..	..	..	391.8	11.3	7.9	..	..	..	..	..
B	168.0	184.7	..	..	..	..	..	352.7	11.8	8.0	..	..	..	..	..
D	263.4	185.6	..	..	..	..	..	459.0	12.6	7.7	..	..	..	..	..
<i>Moradabad</i>															
..	40.0	100.7	307.6	672.0	557.0	442.2	..	2119.5	18.0	15.9	15.8	..	..	..	..



TABLE I.—(continued).

Description of plot	GRM. OF OPIUM DRIED AT 100°C. PER PLOT FROM EACH SUCCESSIVE LANCING								MORPHINE CONTENT OF DITTO						
	1st	2nd	3rd	4th	5th	6th	7th	Total	1st	2nd	3rd	4th	5th	6th	7th
(b) Modified method of lancing, i.e., triple lancing 1917=18.															
<i>Cawnpore</i> Field 50	1	93.9	37.0	32.5	2.2	9.1	..	..	174.7	15.1	13.3	9.9	7.8	8.4	..
	2	122.7	91.5	68.4	2.5	2.2	..	..	287.3	16.7	13.4	9.8	8.4	6.2	..
	3	146.4	74.8	9.1	..	..	..	..	230.3	15.6	10.4	11.1	..	..	..
	4	188.9	45.2	13.3	..	..	..	..	247.4	14.8	11.3	9.4	..	..	..
	5	75.1	29.2	5.3	..	..	..	..	109.8	15.0	12.1	8.3	..	..	..
	6	182.2	34.7	8.8	..	..	..	..	225.7	13.4	10.6	10.2	..	..	..
	7	53.4	20.3	6.0	..	..	..	..	79.7	14.0	10.3	9.0	..	..	..
	8	88.0	17.6	3.5	..	..	..	..	109.1	11.5	9.4	5.6	..	..	..
	9	79.6	34.7	5.1	..	..	..	..	119.4	13.9	11.2	9.1	..	..	..
	10	198.5	49.1	44.3	2.2	4.2	..	..	298.3	16.4	10.9	9.5	6.6	8.0	..
<i>Cawnpore</i> Field 75	..	2	33.1	11.5	2.4	0.6	..	..	47.6	14.1	8.7	6.1	2.6	..	..
Plot	..	5	48.7	4.9	0.9	0.1	..	..	54.6	13.2	7.8	6.0	0.0	..	..
<i>Ankin</i> Plot	..	II	424.0	555.2	253.6	89.0	13.6	..	1335.4	14.0	12.8	11.8	11.0	8.2	..
	..	IV	395.9	397.3	141.2	55.1	18.0	..	1007.5	13.9	13.2	11.1	9.2	7.6	..
<i>Experiments on cultivators' fields.</i>															
<i>Ballia</i>	..	B	612.0	268.9	126.0	71.3	24.2	..	1102.4	12.0	6.7	5.0	..	..	..
	..	D	892.8	325.4	143.4	49.2	25.5	..	1436.3	11.4	6.8	6.2	..	..	..
<i>Dulmau</i> Lala's Field	2	395.6	157.9	34.8	7.5	17.6	1.9	..	615.3	8.3	5.7	4.6	..	..	..
Ditto	..	4	417.5	126.4	36.2	13.4	15.5	1.1	610.1	8.5	6.6	4.8	..	..	..
<i>Bhagwan's</i> Field	..	2	223.7	474.6	218.7	74.1	31.6	5.1	1030.1	11.3	10.0	7.7	..	..	..
Ditto	..	4	221.6	416.2	239.1	65.7	32.8	3.7	980.5	11.1	11.0	6.7	..	..	..
<i>Rae Bareilly</i>	..	B	408.5	195.4	159.0	108.0	27.2	..	898.1	11.3	11.7	8.4	..	..	..
	..	D	345.0	490.0	288.9	52.6	..	..	1176.5	11.4	10.2	8.8	..	..	..
<i>Fyzabad</i>	..	1	430.1	294.0	49.7	..	..	..	773.8	11.8	8.6	7.0	..	..	..
Saktu Teli's Field	..	2	579.2	282.9	17.9	..	..	..	880.0	12.0	8.0	6.0	..	..	..
	..	3	432.0	108.6	10.1	..	..	..	550.7	13.3	7.3	7.0	..	..	..
<i>Patan's</i> Field	..	1	438.5	263.0	..	..	..	..	701.5	12.6	11.0	..	..	..	..
	..	2	647.7	138.9	..	..	..	..	786.6	12.5	8.1	..	..	..	..
	..	3	464.0	91.4	..	..	..	..	555.4	11.8	9.6	..	..	..	..
<i>Ghazipur</i>	..	B	276.2	192.3	62.0	32.8	..	..	563.3	12.3	..	8.3	..	..	..
	..	C	318.6	121.3	62.9	29.2	..	..	532.0	11.2	7.8	8.3	..	..	..
<i>Ghazipur</i> Factory	A	33.1	45.2	75.3	29.8	12.9	4.0	..	200.3	13.6	13.5	12.9	11.0	..	..
	B	84.2	95.0	81.7	11.0	12.9	6.0	..	290.8	13.6	13.8	12.6	..	..	..
	C	16.8	64.8	42.2	16.8	4.0	3.2	..	147.8	13.8	13.2	13.5	11.4	..	..
<i>Lucknow</i>	..	A	229.8	83.1	..	..	..	..	312.9	10.1	7.9	..	..	..	..
	..	B	260.5	79.2	..	..	..	..	339.7	11.3	6.8	..	..	..	..
	..	D	331.6	79.5	..	..	..	..	411.1	7.3	6.6	..	..	..	..
<i>Moradabad</i>	..	..	232.6	394.8	471.9	394.0	217.2	98.2	1808.7	15.8	14.0	12.5	..	..	..

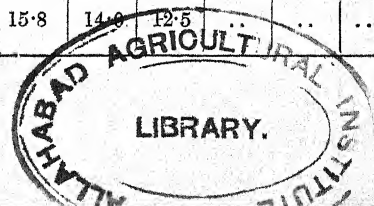


TABLE II.

Showing yield of opium per 1,000 capsules at each successive lancing and morphine content of the same.

Description of plot	GRM. OF OPIUM DRIED AT 100°C. PER 1,000 CAPSULES AT EACH SUCCESSIVE LANCING								MORPHINE CONTENT OF DITTO						
	1st	2nd	3rd	4th	5th	6th	7th	Total	1st	2nd	3rd	4th	5th	6th	7th
(a) Ordinary country method of lancing, 1916-17.															
<i>Cawnpore</i>															
Field .. 55	..	..	..	..	..	..	..	..	14.3	11.3	8.9	2.7	..	..	..
Plot .. 15	..	..	..	..	..	..	..	..	13.9	12.4	6.7	5.6	..	..	..
<i>Sitoli</i>															
Field .. 3	36.2	34.2	14.7	14.8	10.6	..	..	110.5	13.0	10.2	6.4	4.3	2.2	..	..
Immature capsules	15.2	11.1	11.2	5.8	7.0	4.0	..	54.3	8.9	6.4	4.6	1.7	1.2	1.2	..
Mature capsules ..	36.8	38.3	18.8	11.6	25.3	12.6	..	143.4	13.9	9.6	5.8	3.6	2.2	1.6	..
Overmature capsules	24.8	21.1	12.9	9.3	..	..	..	68.1	13.3	10.2	5.0	2.8	..	..	..
Field .. 1	29.6	20.0	20.4	21.3	4.4	..	..	95.7	11.8	8.7	6.7	3.6	1.2	..	..
Field .. 4	16.5	11.7	10.8	..	..	..	..	39.0	13.6	11.7	8.4	..	..	..	..
1917-1918															
<i>Douglas Dale</i>															
Field .. 3c	19.5	23.7	25.3	25.3	9.5	5.0	..	108.3	12.0	7.8	5.6	2.8	1.3	..	..
Field .. 3c	16.4	21.0	23.5	22.6	14.6	4.2	..	102.3	12.2	7.7	6.4	3.5	1.2	..	..
Field .. 3c	20.6	33.8	29.5	18.2	5.6	0.5	..	108.2	13.3	8.9	5.6	2.1	2.8	..	..
Field .. 3c	40.0	40.5	20.9	8.3	8.0	0.6	..	118.3	12.5	6.7	2.8	2.6	2.1	..	..
Field .. 3c	40.2	43.3	25.5	8.7	8.7	3.1	..	129.5	12.5	8.5	3.7	1.5	1.7	..	..
Field .. 1a	37.6	30.0	29.9	42.6	13.1	..	..	153.2	11.3	8.0	4.3	1.4	1.3	..	..
Field .. 1b	29.7	25.9	22.2	24.0	10.1	..	..	111.9	12.0	6.4	2.5	1.4	..	..	..
Field .. I	17.4	27.7	36.1	17.1	7.9	..	..	112.2	14.3	10.5	7.1	4.0	2.4	1.5	..
<i>Ankin</i>															
Plot .. I	69.0	..	..	..	..	..	..	..	15.7	11.1	9.7	..	..	..	..
Plot .. I	..	..	..	..	..	..	..	..	13.6	9.4	..	..	..	..	..
Plot .. III	79.6	..	..	..	..	..	..	..	16.7	14.5	10.5	8.2	..	..	..
Plot .. III	..	..	..	..	..	..	..	..	12.5	10.4	9.0	..	..	..	..
<i>Almora</i>															
Expt. I	21.1	18.0	18.5	13.3	17.8	..	..	88.7	11.5	8.6	5.7	3.5	1.5	..	..
Expt. II	16.5	11.7	10.8	..	..	..	..	39.0	13.6	11.6	8.4	..	..	..	..
<i>Cawnpore</i>															
Field .. 1	33.4	..	..	..	..	..	..	..	14.7	..	..	..	..	..	..
Field .. 2	55.7	..	..	..	..	..	..	..	14.7	..	..	..	..	..	..
Field .. 3	46.6	..	..	..	..	..	..	..	14.9	..	..	..	..	..	..
Field .. 4	40.6	..	..	..	..	..	..	..	13.8	..	..	..	..	..	..
Field .. 5	24.7	..	..	..	..	..	..	..	13.8	..	..	..	..	..	..
Field .. 6	42.0	..	..	..	..	..	..	..	15.0	..	..	..	..	..	..
Field .. 7	31.5	..	..	..	..	..	..	..	15.8	..	..	..	..	..	..
Field .. 8	26.7	..	..	..	..	..	..	..	14.9	..	..	..	..	..	..
Field .. 9	35.4	..	..	..	..	..	..	..	14.1	..	..	..	..	..	..
Field .. 10	38.5	..	..	..	..	..	..	..	14.5	..	..	..	..	..	..

TABLE II—(continued).

Description of plot	GRM. OF OPIUM DRIED AT 100°C. PER 1,000 CAPSULES AT EACH SUCCESSIVE LANCING								MORPHINE CONTENT OF DITTO						
	1st	2nd	3rd	4th	5th	6th	7th	Total	1st	2nd	3rd	4th	5th	6th	7th
(b) Modified method of lancing, i.e., triple lancing, 1917-18.															
<i>Cawnpore</i>															
Field 13 Group .. 1	51.3	10.3	..	..	..	..	..	61.5	10.9	6.2	..	..	..	..	..
.. 2	49.2	16.9	..	..	..	..	..	66.1	12.9	6.0	..	..	..	..	..
.. 2a	61.5	20.5	..	..	..	..	..	82.0	12.8	6.3	..	..	..	..	..
.. 3	55.1	20.4	..	..	..	..	..	75.5	12.7	6.5	..	..	..	..	..
.. 3a	78.0	27.4	..	..	..	..	..	105.4	12.8	7.4	..	..	..	..	..
.. 4	43.1	20.4	..	..	..	..	..	63.5	13.3	7.6	..	..	..	..	..
.. 5	30.1	5.1	..	..	..	..	..	35.2	14.3	9.3	..	..	..	..	..
.. 5a	59.2	11.4	..	..	..	..	..	70.6	14.1	7.1	..	..	..	..	..
Field 74 .. (a)	83.3	22.6	6.9	..	..	..	..	112.8	14.1	7.8	6.6	..	..	..	..
.. (b)	51.0	18.4	6.5	..	..	..	..	75.9	14.2	9.7	5.4	..	..	..	..
.. (c)	70.3	31.6	19.6	..	..	..	..	121.5	14.6	9.7	4.8	..	..	..	..
.. (d)	46.3	10.7	6.0	..	..	..	..	63.0	13.9	10.7	6.6	..	..	..	..
Field 74-75 .. (a)	79.7	27.7	8.2	3.5	..	..	..	119.1	14.0	8.4	5.3	3.3	..	..	..
.. (b)	34.6	8.0	2.2	0.3	..	..	..	45.1	12.2	7.1	4.9	..	..	..	..
Field 12 .. 28-2-18	52.1	35.4	18.5	..	..	..	..	106.0	14.9	10.2	7.0	..	..	..	..
.. 1-3-18	62.1	28.7	15.4	..	..	..	..	106.2	14.5	9.9	6.3	..	..	..	..
.. 2-3-18	60.3	33.2	13.6	..	..	..	..	107.1	14.9	9.4	6.3	..	..	..	..
.. 3-3-18	53.1	31.9	10.9	..	..	..	..	95.9	14.9	9.8	6.2	..	..	..	..
.. 4-3-18	60.1	32.9	9.0	..	..	..	..	102.0	14.6	8.2	6.6	..	..	..	..
.. 5-3-18	61.1	28.9	8.4	..	..	..	..	98.4	14.5	9.5	7.6	..	..	..	..
.. 6-3-18	64.5	24.9	7.5	..	..	..	..	96.9	13.9	8.9	6.4	..	..	..	..
.. 7-3-18	54.7	22.0	9.6	..	..	..	..	85.3	14.2	10.4	6.1	..	..	..	..
.. 8-3-18	51.3	23.0	8.1	..	..	..	..	82.4	13.6	11.0	9.2	..	..	..	..
.. 9-3-18	50.4	21.5	6.5	..	..	..	..	78.4	14.0	8.8	7.7	..	..	..	..
.. 10-3-18	43.2	21.3	8.3	..	..	..	..	72.8	14.2	10.1	7.5	..	..	..	..
.. 11-3-18	45.4	18.7	7.7	..	..	..	..	71.8	14.1	11.6	7.3	..	..	..	..
.. 12-3-18	41.5	23.4	7.5	..	..	..	..	72.4	14.8	11.9	6.8	..	..	..	..
.. 13-3-18	33.0	21.9	4.9	..	..	..	..	59.8	15.3	11.7	9.1	..	..	..	..
.. 14-3-18	33.0	13.7	3.1	..	..	..	..	49.8	15.1	11.3	8.5	..	..	..	..
.. 15-3-18	27.5	8.0	2.3	..	..	..	..	37.8	14.8	11.2	7.4	..	..	..	..
.. 16-3-18	24.4	6.3	1.6	..	..	..	..	32.3	14.6	12.4	8.8	..	..	..	..
.. 17-3-18	19.4	7.9	1.6	..	..	..	..	28.9	15.0	13.9	6.6	..	..	..	..
.. 18-3-18	18.1	5.3	2.1	..	..	..	..	25.5	15.0	12.3	..	..	..	..	..
.. 19-3-18	12.3	2.6	3.2	..	..	..	..	18.1	15.7	14.6	..	..	..	..	..
Field .. 38	97.2	15.5	..	..	..	..	..	112.7	15.0	7.1	..	..	..	..	..
.. 39	65.4	20.0	..	..	..	..	..	85.4	15.3	9.5	..	..	..	..	..
Field 73 Plot .. 5	69.2	10.6	..	..	..	..	..	79.8	12.7	6.3	..	..	..	..	..
.. 7	54.4	10.0	..	..	..	..	..	64.4	13.4	6.9	..	..	..	..	..
Field 50 Plot .. 1	48.4	..	..	..	..	..	..	..	14.7	..	..	..	..	..	..
.. 2	52.4	..	..	..	..	..	..	..	15.7	..	..	..	..	..	..
.. 3	45.4	..	..	..	..	..	..	..	14.7	..	..	..	..	..	..
.. 4	46.7	..	..	..	..	..	..	..	13.7	..	..	..	..	..	..
.. 5	29.5	..	..	..	..	..	..	..	14.8	..	..	..	..	..	..
.. 6	44.3	..	..	..	..	..	..	..	12.5	..	..	..	..	..	..
.. 7	18.3	..	..	..	..	..	..	..	13.9	..	..	..	..	..	..
.. 8	23.1	..	..	..	..	..	..	..	12.6	..	..	..	..	..	..
.. 9	29.1	..	..	..	..	..	..	..	13.4	..	..	..	..	..	..
.. 10	57.2	..	..	..	..	..	..	..	16.7	..	..	..	..	..	..
<i>Ankin</i>															
Plot .. II	92.4	..	..	..	..	..	..	..	14.0	13.2	11.4	..	..	..	..
.. II	..	..	..	..	..	..	..	..	12.3	11.6	..	..	..	..	..
Plot .. IV	98.7	..	..	..	..	..	..	..	13.9	13.2	10.1	..	..	..	..
.. IV	..	..	..	..	..	..	..	..	13.2	11.0	..	..	..	..	..
<i>Almora</i>															
Expt. .. I	59.8	26.7	8.8	4.1	..	..	..	99.4	10.9	5.3	2.1	1.9	..	..	..
.. II	29.1	11.2	6.8	..	..	..	..	47.1	13.2	6.8	2.3	..	..	..	..

It is thus perfectly clear that the morphine content of the opium decreases rapidly in the subsequent lancements with the cultivator's method of lancing. In general, the yield of opium per capsule is practically as high at the second as at the first lancing, but after that the yield also falls off rapidly. When more than one cut is applied at each lancing the yield falls off rapidly at the second and subsequent lancements.

As a result of this work we were able to point out the reason why Indian opium was inferior in morphine content to that produced in Turkey. In the latter country each capsule only receives one incision. That incision corresponds in morphine content to the produce of opium of the first incision as carried out in India.

As explained already (p. 7), the Indian cultivator mixes the whole of his opium from all the successive lancements, hence the morphine content of the mixed opium is naturally much lower than that of Turkish opium.

Great interest attaches to the discovery of the substance which takes the place of the morphine in the later lancements. Suffice it to say here that the percentage of total nitrogen in the opium of the successive scarifications remains practically constant. It seemed quite probable that in the place of morphine some other alkaloid, *e.g.*, codeine, might be produced in the later lancements. An estimation of the amount of morphine, narcotine and codeine in the opium of four successive lancements gave the following results:—

No. of lancements			PERCENTAGE OF ALKALOIDS IN DRY MATTER			Total of morphine, narcotine and codeine
			Morphine	Narcotine	Codeine	
1st	..	..	11.18	6.85	2.25	20.28
2nd	..	..	9.28	6.34	2.71	18.33
3rd	..	..	6.38	4.18	2.94	13.50
4th	..	..	3.41	3.36	3.64	10.41

It thus appears that narcotine falls off in the successive lancements in a somewhat similar manner to morphine, though the fall is not so rapid. Codeine shows no falling off in the successive lancements but rather a slight increase. The experiment is typical of other similar ones as regards morphine and codeine. The increase in the case of codeine, however, is only slight and not at all sufficient to compensate for the falling off in the morphine content. Narcotine has not, however, always given the same results. The matter is still under

investigation but our experiments so far lead us to the conclusion that some non-alkaloidal nitrogenous substance is produced in the later lancements in place of morphine.

It might be suggested that the falling off in morphine content is due to the fact that the first lancing is carried out at a stage of ripeness of the capsule at which morphine is most concentrated in it, and that during the later lancements the capsule is ripening off with an accompanying decrease in morphine content. That this is not the case we have definitely established by lancing capsules in all stages from a few days to one month old. Though the opium yield varies, yet the morphine content of the first lancing remains more or less the same at whatever stage of ripeness the capsule is lanced. These experiments will be fully described later.

*Experiments to determine if the morphine content of the opium from the second and subsequent lancements can be modified by varying the intervals of time between the successive lancements.*

#### *1917-1918 experiments.*

As already stated, the Indian cultivator usually leaves an interval of 3 days between the successive lancements of his capsules. We have experimented with the following intervals of time between the successive lancements, (1) 4 hours, (2) 1 day, (3) 2 days, (4) 3 days, (5) 4 days and (6) 5 days.

*Details of the experiments.* A field was divided into 3 plots. In each plot 1,000 plants, possessing only one capsule each,\* were then selected, the capsules being ripe for lancing as judged by the cultivators. On each plant a piece of black cloth was tied to simplify the finding of them during the course of the experiment.

At 9 a.m. on 18th March, 1918, each capsule was lanced with a single cut of a 4-bladed knife as in the usual country way. At 1 p.m., the usual time of lancing in India, each of these capsules received a second cut to the right of the first cut. At 5 p.m. each capsule received a third cut again to the right of the second cut. Next morning at 6 a.m. the opium from the left-hand cuts—representing the first lancing—was collected by one set of men. The opium from the middle cuts, equivalent to the second lancing, was collected by another set of men and the opium from the right hand cuts, representing the third lancing, was collected by a third

\* This was necessary as it will be shown later that where a plant produces more than one capsule its younger capsules produce opium of inferior morphine content.



set of men. The collections on each plot were kept entirely separate, so that the whole experiment was triplicated. All the plants yielded opium from the first and second cuts, but in plots 1 and 2 some of the plants yielded no opium from the third cuts. On the 21st March, 1918, a fourth, fifth and sixth cut was put on each head at 9 a.m., 1 p.m. and 5 p.m. respectively as before and the produce of each cut collected separately at 6 a.m. next morning. The number of heads yielding latex and its amount were very small.

The samples were allowed to dry at the temperature of the laboratory, being spread out in a thin layer. Each sample was torn into small pieces as soon as possible, and when dry enough these were ground to a powder which contained 92 to 95 per cent dry matter. The morphine was determined in 8 gm. of each sample by the method of the *British Pharmacopæia* (1914) and calculated to dry opium by allowing for the loss in weight found by heating a further 2 gm. portion of the sample in the water oven for 24 hours.

The following table sets out all the results:—

Date		Plot 1			Plot 2			Plot 3		
		9 a.m. lancing	1 p.m. lancing	5 p.m. lancing	9 a.m. lancing	1 p.m. lancing	5 p.m. lancing	9 a.m. lancing	1 p.m. lancing	5 p.m. lancing
18th March 1918 ..	No of plants yielding opium ..	1000	1000	930	1000	1000	971	1000	1000	1000
	Total dry opium gm. ..	36.5	20.6	10.8	34.8	21.6	9.7	41.1	21.0	22.8
	Morphine % on dry opium ..	17.6	13.7	13.5	18.4	14.9	12.3	18.1	13.1	14.9
21st March 1918 ..	No. of plants yielding opium ..	167	102	317	150	75	36	140	50	84
	Total dry opium gm. ..	3.6*	0.8*	0.8*	..	..	..	..	..	..
	Morphine % on dry opium ..	6.9	..	..	..	..	..	..	..	..

\* Represents total yields in plots 1, 2 and 3 as all collected together owing to small quantity. Morphine was estimated in the 3.6 gm. dry matter available by taking proportionate amounts of reagents.

A similar experiment was carried out during the season 1917-18 at Douglas Dale near Naini Tal (elevation 4,000 feet). Owing to lack of facilities in the hills, and to a small area of poppy, the experiments could not be duplicated. 641 single-capsuled plants were selected, and were distinguished by yellow cloth labels. The remaining details of the experiments were exactly the same as those of the experiment already described above, but in addition the experiment was carried out for a third day's lancing, at the end of which the heads had of course received 9 cuts each.

The table sets out the results.

Date of lancing			Hour of lancing	Number of plants yielding opium	Total dry opium grm.	Morphine percentage on dry matter	Grm. of dry opium per 1,000 capsules
8th April 1918	..	..	9 a.m.	641	19.4	13.1	30.3
			1 p.m.	637	14.9	10.1	23.2
			5 p.m.	631	5.8	7.1	9.1
11th April 1918	..	..	9 a.m.	613	7.8	7.3	12.1
			1 p.m.	607	7.3	4.9	11.4
			5 p.m.	614	3.8	3.5	5.9
14th April 1918	..	..	9 a.m.	355	5.9	3.1	9.2
			1 p.m.	115	1.3	..	2.0
			5 p.m.	Not lanced as so few plants yielded at 1 p.m.			

The results in the hills and at Cawnpore seem to differ somewhat. In considering them it should be mentioned that the same pure race of poppy was used for both sets of experiments. The total yield of dry opium per 1,000 capsules is more or less the same at both places and in each instance it diminishes with the successive lancements. As regards the morphine content of the opium, we see that the hill experiment shows about the same falling off in the morphine content of the successive lancements, all of which are carried out on the same



day, as we find takes place in the opium of the successive lancements as carried out by the cultivator. Moreover at the second day's lancing (11th April) the morphine content has fallen lower still and a progressive fall again takes place in the morphine content of opium from the three lancements of that day. In the case of the experiment at Cawnpore the morphine content of the opium collected from the 9 a.m. incision is extraordinarily high, about 18 per cent in each of the triplicate experiments. The opium from the 1 p.m. and 5 p.m. lancements is however still very rich in morphine and the opium from the 5 p.m. lancing does not show the big drop in morphine content below that of the opium from the 1 p.m. lancing, which one would have been led to expect from the hill experiment. During the same season a very large number of morphine estimations of the opium from the first lancements of this same pure race of poppy from surrounding areas were made and even from capsules on the same field. None of these gave 18 per cent morphine and rarely reached 16 per cent. It seems significant that each of our triplicate experiments should produce about 18 per cent morphine. It seemed possible that the time of day at which the incision is made might have some effect on the morphine content of the opium. Experiments showed however that this is not the case. (See Part V, page 57.)

In the following experiments the interval of time between the successive lancements was one day or more. There were two sets of these experiments.

(A) in which the capsules received only one cut at each lancing as in the ordinary country method.

(B) in which the capsules received three cuts at each lancing.

(A). 4,000 single-capsuled plants, ripe for lancing, were selected by means of differently coloured cloth labels. Four groups I, II, III, IV, each of 1,000 capsules were marked off. A group was not confined to a particular portion of the field but each group was uniformly distributed over the whole area. This would tend to rule out any possible effect of inequalities of crop on different portions of a field. It was proposed to lance Group No. I with one day's interval between each lancing, but, by an oversight, the third lancing was not done on the third day but on the sixth day. Group No. II received its second lancing on the third day and its third lancing on the sixth day instead of the fifth day. Group No. III received its second lancing on the fourth day, and its third lancing on the seventh day, that is, after three days' intervals as proposed. Group No. IV received its second lancing on the sixth day and its third lancing on the 11th day, that is, correctly, after five days' intervals.

The results are summarized in the following table :—

No. of group	No. of lancing	Date of lancing	Interval in days between lancings	No. of capsules yielding opium	Weight of dry opium	Percentage of morphine in dry opium
I	1st	11-3-18	..	1,000	31.1	14.5
	2nd	12-3-18	1	973	13.7	10.0
	3rd	16-3-18	4	468	4.1	7.0
II	1st	11-3-18	..	1,000	28.5	15.4
	2nd	13-3-18	2	977	30.4	10.3
	3rd	16-3-18	3	498	10.9	6.4
III	1st	11-3-18	..	1,000	47.7	15.3
	2nd	14-3-18	3	987	27.1	8.9
	3rd	17-3-18	3	..	3.5	4.7
IV	1st	11-3-18	..	1,000	36.1	14.8
	2nd	16-3-18	5	913	24.4	8.6
	3rd	21-3-18	5	219	1.8	3.9

(B). This was an experiment on similar lines to the above, but the heads received three vertical cuts with the knife at each lancing. Unfortunately the yield of opium at the second lancing was too small for analysis in one case. The results are set out in the table.

No. of group	No. of lancing	Date of lancing	Interval in days between lancings	No. of capsules yielding opium	Weight of dry opium	Percentage of morphine in dry opium
I	1st	8-3-18	..	1,000	56.8	13.9
	2nd	9-3-18	1	130	1.2	..
II	1st	8-3-18	..	1,000	69.0	14.5
	2nd	10-3-18	2	136	5.6	9.3
III	1st	8-3-18	..	1,000	71.4	14.0
	2nd	11-3-18	3	200	4.6	7.5
IV	1st	8-3-18	..	1,000	71.4	15.1
	2nd	12-3-18	4	664	26.2	8.5

#### 1918-1919 experiments.

In the season 1918-19 further experiments were carried out to determine the influence of interval of time between the successive lancements on the yield and morphine content of the latex.

Six groups each of 1,000 terminal capsules at the correct stage of lancing were selected. Each group was labelled with labels of a distinctive colour to

make recognition easy. Each group then received three successive lancements at intervals of 2, 4, 8, 24, 48 and 96 hours between each lancing for each respective group. The lancing in each case consisted of one vertical incision with a 4-bladed knife. The results are summarized in the table.

*Table showing effect of intervals of time between each successive lancing on the yield and morphine content of the latex.*

Group	Interval between successive lancements	No. of lancing	Date of lancing		No. of cap. sule lanced	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium	Grm. morphine recovered per 1,000 capsules
I	2 hours ..	1st	22-3-19	1 p.m.	1,000	47.33	13.4	6.35
		2nd	"	3 p.m.	1,000	21.67	11.0	2.38
		3rd	"	5 p.m.	1,000	14.58	8.2	1.20
		Total	..			83.63		9.93
II	4 hours ..	1st	22-3-19	9 a.m.	1,000	14.59	12.9	1.88
		2nd	"	1 p.m.	1,000	21.17	11.0	2.33
		3rd	"	5 p.m.	1,000	9.75	8.6	0.84
		Total	..			45.51		5.05
III	8 hours ..	1st	22-3-19	9 a.m.	1,000	16.08	14.0	2.25
		2nd	"	5 p.m.	1,000	24.23	12.2	2.96
		3rd	23-3-19*	9 a.m.	1,000	19.54	7.3	1.43
		Total	..			59.85		7.64
IV	24 hours ..	1st	22-3-19	1 p.m.	1,000	35.18	12.2	4.29
		2nd	23-3-19	1 p.m.	1,000	25.85	8.5	2.40
		3rd	24-3-19	1 p.m.	1,000	13.91	6.0	0.83
		Total	..			74.94		7.52
V	48 hours ..	1st	22-3-19	1 p.m.	1,000	29.29	12.0	3.51
		2nd	24-3-19	1 p.m.	1,000	37.66	8.8	3.31
		3rd	26-3-19	1 p.m.	1,000	18.02	3.5	0.63
		Total	..			84.97		7.45
VI	96 hours ..	1st	22-3-19	1 p.m.	1,000	36.12	11.5	4.15
		2nd	26-3-19	1 p.m.	1,000	34.59	8.2	2.84
		3rd	30-3-19	1 p.m.	1,000	14.53	5.3	0.77
		Total	..			85.24		7.76

\* This lancing should have been done at 1 a.m. but it was postponed till 9 a.m.

In each case the collection was made at 8 a.m., next day.

Taking the two years' results together, it seems clearly indicated that provided the interval between the successive lancing is one day or more there is no difference in the rate at which the percentage of morphine diminishes in the opium from each successive lancing. When the interval of time between the successive lancements is 8 hours or less then the rate at which the percentage of morphine diminishes in the opium from each successive lancing is appreciably lessened.

Further, there is an indication in the tables that after receiving the first lancing, the capsules require a certain minimum interval of time before the second lancing in order that they may give a free yield of latex at the second lancing.

Thus with experiment A 1917-18 (single cuts at each lancing), the yields of dry opium per 1000 capsules at the first lancing were 31.2, 28.5, 47.7 and 36.1 gm. in Groups I, II, III and IV respectively. In these groups the second lancing was carried out after intervals of 1, 2, 3 and 5 days respectively and the yields of dry opium were 13.7, 30.4, 27.1 and 24.4 gm. It would seem that one day's interval between the first and second lancing is not sufficient to enable the plant to fully regain its latex-producing power. In the case of experiment B, where at each lancing 3 cuts were applied it would appear that the plant required longer to recover from the effect of the first cut, as the yields of the first lancements were, per 1,000 plants, 56.8, 69.0, 71.4 and 71.4 gm. of dry opium in Groups I, II, III and IV respectively. In Groups I, II and III lanced for the second time after 1, 2 and 3 days' intervals respectively the yield of opium at the second lancing was almost negligible, but in group IV in which the second lancing took place after 4 days the yield was 26.2 gm. In this experiment it is also of interest to note the number of plants yielding latex at the second lancing. In Groups I, II, III and IV this number was 130, 136, 200 and 664 respectively. This also indicates that the second lancing should not follow too closely on the first or the yield of the second lancing will suffer considerably.

The rate at which the morphine percentage falls off in each successive lancing is appreciably affected by the length of the incisions made on the capsules. Data on this point are given later in Section V of this Memoir.

#### CONCLUSIONS.

1. The opium obtained at the first lancing of a poppy capsule is richer in morphine than that of the succeeding lancements. Each successive lancing gives opium of progressively decreasing morphine content. Hence in work

on opium it is essential to keep for separate examination the product of each successive lancing. This point has been overlooked in certain work carried out in the past on opium.

2. The decreasing morphine content of the opium of the later lancements is not counterbalanced by a rise in codeine or narcotine content.

3. The interval of time between the successive lancements does not affect the rate of the falling off in the morphine content whether the interval be one or five days. When only four to eight hours have elapsed between the successive lancements the falling off in morphine content is not so rapid. This may be accounted for by supposing that a few hours are not sufficient to exhaust the latex already in the capsule by one incision. Work described later proves that this flow continues for at least 16 hours from one cut. If then the second cut is applied within a few hours of the first the latex exuding from this second cut will be partly latex which was already in the capsule and therefore richer in morphine and which with one day's interval between the lancements would have all been exuded at the first lanced surface.

4. The rate of falling off can however be appreciably diminished by decreasing the length of the incision made on the capsule and thereby decreasing the yield of latex at each incision. Much more information on this point is given later.

5. As regards the proportion of the total out-turn obtained at each lancing the figures show that no general average can be stated. Table II expresses yield per 1,000 capsules. It indicates that in general each capsule gives its maximum yield of latex at the first lancing and that the yield falls off with each successive lancing.

Table I (p. 9) deals with the out-turn of each successive lancing. The second lancing frequently yields more opium than the first. The first lancing is a very variable quantity. It may form anything from 10 to over 40 per cent of the total out-turn. If the cultivator lances his field too early he will of course find only a few heads ready for lancing, and in this case his first lancing will form a much smaller proportion of the total out-turn than if he had delayed his first lancing until such time as the bulk of his capsules were ready for the first lancing. The relative yields of opium at each lancing will also vary with the luxuriance of the crop. A heavy crop of big capsules may give almost equal yields at each of the first three or four successive lancements. Moreover certain weather<sup>1</sup> conditions may frequently cause an abnormally high yield in a later lancing.

<sup>1</sup>*Memoirs of the Department of Agriculture in India, Chemical Series, Vol., VI, No. 2.*



#### PART IV.

##### THE VARIATIONS IN RATE OF FLOW AND MORPHINE CONTENT OF THE LATEX AT DIFFERENT PERIODS OF TIME AFTER INCISION.

There is apparently nothing in the literature bearing on the rate of flow of the latex or on the changes in its alkaloidal content at different intervals of time after incision of the capsules. This is strange because the time which has elapsed between the incision of the capsule and the collection of opium has a very marked influence on the morphine content. Moreover work of this nature might have a very important bearing on the study of the function of the alkaloids in the plant.

##### *Experiments of 1916-17 and 1917-18.*

A number of experiments were carried out and the plan of each was practically the same. A number of capsules ready for lancing were lanced at 2 p.m. the time usually chosen by the cultivator. One set of men performed the lancing, and accompanying them was another set of men who collected the latex within a few seconds of lancing. By 5 p.m. the same evening the lanced surface had exuded some more latex and this was collected and kept separate from the first collections. By 6-30 a.m. next morning more latex was found to have exuded from the same surface and it was similarly collected. In some experiments however the instantaneous collection and in others the 5 p.m. collection was omitted. The number of capsules lanced was counted. In addition the dry matter of the opium of each collection and also its morphine content were determined. The results are all summarized in the table.

In most of the experiments the capsules were lanced in the usual<sup>1</sup> Indian way. A few experiments however were carried out in which three simultaneous cuts were made on the capsule at each lancing instead of the one cut usually made by the cultivator. Experiments made in this way are therefore distinguished under a separate heading in the table.

<sup>1</sup> See Part II, p. 7

No. of ex-periment	Locality of experiment	No. of lanc-ing	Date of lanc-ing	YIELD OF OPIUM						% OF MORPHINE IN OPIUM			
				INSTANTANEOUS COLLECTION 2 p.m.		5 p.m. COLLECTION		6-30 a.m. COLLECTION next day		Total dried opium grm.	No. of capsules yield-ing latex	Yield of dry opium per 1000 sules grm.	In-stan-tan-eous collec-tion at 2 p.m.
				Grm. dried opium	% of total yield	Grm. dried opium	% of total yield	Grm. dried opium	% of total yield				
1	Almora	1st	20-4-17	35.05	24.8	42.45	30.0	63.80	45.2	141.30	8653	16.32	15.2
2	Do.	2nd	24-4-17	29.30	20.3	82.35	57.0	22.95	15.8	144.60	8653	16.71	12.3
3	Do.	3rd	27-4-17	33.10	31.3	53.50	50.7	19.00	18.0	163.60	8653	12.20	8.1
4	Do.	4th	30-4-17	Latex	liquid	48.85	..	14.85	..	..	8653	..	..
5	Do.	5th	3-5-17	..	..	28.20	..	11.05	..	..	8653	..	..
6	Do.	1st	26-4-17	5.75	47.5	No collection	..	6.35	52.5	12.10	2088	1.40	11.2
7	Do.	2nd	29-4-17	5.70	55.3	..	..	4.60	44.7	10.30	2088	1.19	10.4
8	Do.	3rd	2-5-17	4.40	60.7	..	..	2.85	39.3	7.25	2088	0.84	7.4
9	Cawnpore	1st	12-3-17	10.69	25.3	..	..	31.58	74.7	42.27	1769	23.90	16.0
10	Do.	2nd	15-3-17	5.60	46.6	..	..	1.30	10.9	12.02	..	6.81	8.1
11	Do.	3rd	19-3-17	3.14	38.1	..	..	5.10	61.9	8.24	..	4.66	4.6
12	Do.	1st	19-3-17	11.00	49.2	No collection	..	11.40	50.8	22.40	..	..	15.0
13	Do.	2nd	22-3-17	5.02	54.3	..	..	4.22	45.7	9.24	..	..	13.6
14	Do.	1st	23-3-17	9.97	29.7	..	..	23.60	70.3	33.57	..	..	9.1
15	Do.	2nd	20-3-17	6.60	31.8	..	..	14.40	68.2	21.00	..	..	7.6
Modified method of lanc-ing, i.e., triple lanc-ing (1917-18).													
16	Do.	1st	19-3-18	21.09	41.9	17.31	34.4	11.94	23.7	50.34	2725	18.47	15.4
17	Do.	1st	20-3-18	58.62	56.5	28.11	19.4	25.03	24.0	103.76	5909	17.56	15.3
18	Do.	2nd	23-3-18	5.31	44.6	3.58	30.1	3.00	25.3	11.89	5909	2.01	10.5
19	Do.	1st	20-3-18	35.41	52.0	23.93	35.1	8.79	12.9	68.13	5857	11.63	19.5
20	Almora	1st	22-4-18	21.90	55.6	6.10	15.5	11.40	28.9	39.40	2400	16.40	10.4

Modified method of lanc-ing, i.e., triple lanc-ing (1917-18).



*1918-19 experiments.*

In the season 1918-19 more detailed experiments were carried out on these lines both at Cawnpore and in the hills at Douglas Dale.

At each place 5,000 terminal capsules were selected and labelled. These were then lanced at 9 a.m. in the ordinary Indian fashion, *i.e.*, by means of a single vertical cut with a four-bladed knife. The latex flowing out of the incision was collected immediately, *i.e.*, within one minute of the time of making the incision. At 12 noon, 3 and 6 p.m. and again next morning at 6 a.m. the latex was collected from the same incised surface. The capsules were again lanced for a second and third time both at Cawnpore and at Douglas Dale. At each lancing the opium was collected in fractions as described at the first lancing. (See Table on page 26.)

It would thus seem that usually about half the latex yielded on incising a capsule exudes within a minute after making the incision, although in a number of cases only about 30 per cent was obtained in the first minute.

The tables shew that the latex first flowing from the cut surface is the richest in morphine and that its morphine content falls off as the flow continues. It is moreover interesting to note that on again lancing the same capsule for a second time the morphine content of the latex of the first runnings from the second lancing is about the same as that of the last runnings of the previous lancing. This would indicate that morphine is stored in the capsule. When the capsule is lanced the latex first flowing out would therefore be richest. As the flow continues the latex would be flowing in from parts of the plant below the capsule where it is less concentrated in morphine. It is difficult to account for the gradually diminishing morphine content of the latex in any other way.

Incidentally these experiments tend to disprove the theory that morphine does not occur as such in the plant. Thus Winterstein and Trier<sup>1</sup> have suggested that morphine occurs as pseudo-morphine in the plant, and that morphine is produced from this in contact with the air by the agency of a *reductase* in the latex. Also True and Stockberger<sup>2</sup> concluded from certain experiments that alkaloids do not exist as such in the poppy plant, but appear as products of the action of *oxidase* on the constituents present in the latex in presence of oxygen. On the other hand Goris and Vischniae<sup>3</sup> in referring to statements met with that morphine is not present in poppy latex but that it is gradually formed in opium by a fermentative process, remark that this is untrue. They found in experiments in 1913 that opium contained

<sup>1</sup> Winterstein und Trier. Die Alkaloide, p. 7

<sup>2</sup> Amer. J. Botany., 1916, 3, 1

<sup>3</sup> Bull. Sci. Pharm. 1915.

*Yield and composition of opium from 5,000 heads lanced at 9 a.m., the later being removed at regular intervals after incision.*

Locality of experiment	No. of lancing	Date of lancing	YIELD OF OPIUM										PERCENTAGE OF MORPHINE IN DRY OPIUM						
			9 A.M., i.e., INSTANTANEOUS COLLECTION		12 noon		3 P.M.		6 P.M.		6 A.M. (NEXT MORNING)		Total grm. dry opium	Yield of dry opium i.e., per 1000 capsules grm.	9 a.m. i.e., instantaneous	12 a.m.	3 p.m.	6 p.m.	6 a.m.
			Grm. dry opium	Per-centage of total yield	Grm. dry opium	Per-centage of total yield	Grm. dry opium	Per-centage of total yield	Grm. dry opium	Per-centage of total yield	Grm. dry opium	Per-centage of total yield							
Cawnpore ..	1	20-3-19	74.04	56.1	38.66	29.3	..	..	..	..	19.28	14.6	131.98	26.40	13.7	11.3	..	..	10.1
	2	23-3-19	62.90	55.8	38.02	33.7	..	..	..	..	11.89	10.5	112.90	22.58	10.5	9.4	..	..	7.1
	3	26-3-19	24.80	45.9	23.11	42.7	..	..	..	..	6.17	11.4	54.08	10.82	7.1	5.6	..	..	4.5
Douglas Dale ..	1	13-4-19	55.37	49.1	52.43	46.5	2.80	2.5	1.91	1.7	0.28	0.2	112.79	22.56	12.3	11.0	10.9	10.1	..
	2	16-4-19	39.60	54.0	27.39	37.6	2.57	3.5	2.72	3.7	0.85	1.2	73.33	14.67	9.7	7.8	7.7	5.9	..
	3	19-4-19	40.30	50.5	33.30	41.7	1.20	1.5	1.10	1.4	3.91	4.9	79.81	15.96	7.3	5.7	..	..	5.5

on the evening of the day of collection 16 per cent of morphine in the dry matter. In an experiment in the following year a yield of 18 per cent morphine calculated on the dry matter was obtained from latex of Turkish opium poppy. The latter sample analysed a year later gave 17.7 per cent morphine. Both samples of opium were produced at Etrechy in France.

#### CONCLUSIONS.

1. The figures given in the previous table establish quite definitely the fact that when a capsule is lanced, the latex which first flows contains the highest percentage of morphine calculated on the dry matter of the latex. As the flow of latex continues the morphine content, reckoned on the dry matter, diminishes considerably. There is one case, Serial No. 6, in the first table in which we find an exception. In that case the latex collected immediately after incision of the capsule contained only 11.2 per cent of morphine in its dry matter. No collection was made again before 6 a.m. next morning. The dry matter of the latex then collected contained 13.0 per cent morphine. It is possible however that this exceptional result may be connected with the fact that in that particular experiment all the leaves had been stripped off the plants two days before the capsules were lanced.

In one other case Serial No. 10 there is another apparent exception. The difference there observed however is within the experimental error of the method of morphine analysis used (*B.P.* 1914).

2. As regards the rate of the flow of the latex this seems to vary considerably, but it will be seen that between 22.6 and 60.6 per cent of the total reckoned in dry matter was exuded within the first five to ten seconds after incision of the capsule.

3. As regards the practical side of this work, it is seen that the ideal way to get opium rich in morphine is to take the latex exuding in the first few seconds from the surface of capsules which have been lanced for the first time. Quite possibly also, if this latex first flowing were removed immediately, a greater total flow of latex would be obtained per capsule if a second collection were made next morning, because the coagulation of the latex around the incised surface must tend to prevent a free flow.

#### PART V.

##### THE INFLUENCE OF VARIATIONS IN THE METHOD AND TIME OF LANCING ON THE YIELD AND COMPOSITION OF THE OPIUM.

The method of lancing poppy capsules varies in the different opium producing countries. It will be of interest if we first shortly describe these methods.

The Indian method has been fully described in Part II above.

The knife \* used consists of 3 or 4 parallel pointed blades set about one-twentieth of an inch apart. The capsule is lanced vertically either from below upwards or from the top downwards. The lancing is carried out after midday and the opium is collected early next morning. For this purpose an iron scoop is used, the opium being scraped off the surface of the capsule by means of it. Each capsule is lanced as long as it will yield latex at intervals of two to three days between each lancing. We have seen as many as 8 lancements per capsule. The opium is kept in earthen pots for several weeks when it is taken over by Government officials. It then contains 65-75 per cent dry matter.

*Persia.* Vertical incisions are made as in India. The "*Encyclopædia Britannica*" states that in some districts vertical cuts with diagonal branches are made. We are uncertain however about the kind of knife used. It would appear that each capsule only receives one lancing. The juice is collected and conveyed to the market in copper vessels. There it is manipulated to suit the tastes of buyers in Hong Kong and London. For home consumption in Persia 20 per cent of foreign matter is added to the crude opium after its volume has been reduced to one sixth by evaporation. This foreign matter consists of the resin of *Penæa mucronata* and extract of poppy heads.<sup>1</sup>

*Egypt*<sup>2</sup>. The capsules are slit twice transversely at the same time and it appears that no subsequent lancements are taken.

The opium is placed on a leaf in the sun to harden.

*Asia Minor and the Balkans.* The method of lancing in the Balkans and Asia Minor seems to be unique and consists in making a spiral incision completely round the capsule apparently with a single-bladed knife. No subsequent lancing is carried out. The incision is carried out in the afternoon and the latex is scraped off next morning and allowed to dry in masses in the shade for several days.

*China.* A four-bladed<sup>3</sup> knife apparently like the Indian knife is used for lancing. A horizontal cut is made daily across the middle of the capsule, each succeeding cut being parallel to the first. As many as ten cuttings may be made on each capsule. Apparently the opium of each successive lancing is mixed together. The collected latex is poured into a kettle and boiled over

\* Such a knife is figured in the *Encyclopædia Britannica*, 11th Edition, Art. on Opium.

<sup>1</sup> Thorpe, *Dict. of Applied Chem.*, Vol. IV, Art. on Opium, p. 16.

<sup>2</sup> *Pharm. Jour.*, (ii) 4, 199.

<sup>3</sup> "Die Opiumzucht in Norden Chinas" van Itallie und Kerbosch. *Archiv der Pharmazie*. 248, 1910, p. 614.

a straw fire. The foam which forms is taken off and the residue on cooling forms a black sticky mass which is sold without further preparation.

*Germany.* In the German experiments<sup>1</sup> a knife was used having three blades 4 mm. apart whose points projected 2 mm. and each capsule received two horizontal cuts with this knife on the day of lancing one below the other, the cuts extending one-third to one-fourth the way round the capsule. Thus each capsule received six parallel horizontal cuts each about 4 mm. apart. Subsequent lancements were rarely carried out.

*Australia*<sup>2</sup>. Two horizontal incisions are made half-way round the capsule and successive lancements are made until the capsule is exhausted just as in India. The produce of all the lancements is apparently mixed together.

*France.* The "*Encyclopædia Britannica*"<sup>3</sup> states that at Clermont Ferrard the latex is evaporated by artificial heat immediately after collection, but we have been unable to discover the details of the method of lancing.

#### EXPERIMENTAL.

We have carried out large numbers of experiments in which we modified the method of lancing as used in India. The Indian cultivator is convinced that his method is the best, and our work supports his judgment. On the theoretical side we are unable to find anything in the literature relating to the structure of the lactiferous system which would enable us to decide the probable relative advantages of vertical *versus* lateral incisions. Thoms<sup>4</sup> states that he finds that if the capsules are lanced length-wise, *i.e.*, vertically as in India, the yield of opium is smaller than if they are lanced transversely. He states that this is only natural because more latex vessels are cut in the latter system. He gives no authority for this statement however. His evidence consists of one or two small experiments. In one of these he lanced 95 capsules in the Indian way and obtained 0.80 grm. opium, whereas from 98 lanced transversely he obtained 1.33 grm. In another experiment the former method gave 0.55 grm. opium from 108 capsules and the latter 1.17 grm. from 102 capsules.

Our experiments on different methods of lancing have been carried out on a large scale and may be summarized under various heads.

1. Comparison of Turkish method of spiral lancing *versus* the Indian method.

<sup>1</sup> Thoms *Über Mohnbau und Opiumgewinnung. Ber der Deutsch. Pharm. Gesellschaft* Berlin 1907, p. 14.

<sup>2</sup> Thorpe, *Dict. of Applied Chem.*, Vol. IV, Art. on Opium, p. 18.

<sup>3</sup> 11th edition.

<sup>4</sup> *Loc. cit.*, p. 14.



2. Comparison of transverse incisions *versus* the usual Indian vertical incisions.
3. Comparison of the Indian method of lancing, *i.e.*, one vertical cut with a three or four-bladed knife *versus* two and three such vertical cuts at each lancing and also *versus* five or six cuts at one lancing with no subsequent lancements.
4. Comparison of the Indian method of lancing, *i.e.*, one vertical cut with a three- or four-bladed knife *versus* the same method in which the length of the cut was only one-third as great and even less.
5. The effect of the number of blades in the knife using the ordinary Indian method of lancing. We used knives having two, four and six blades for comparison.
6. The effect of lancing in the afternoon and collecting in the morning as in the usual Indian way *versus* lancing in the morning and collecting in the evening.

1. COMPARISON OF TURKISH METHOD OF SPIRAL LANCING  
WITH THE INDIAN METHOD.

This experiment was carried out in 1916-17 at Cawnpore in a field measuring 0.5 acre. The seed used was of a pure race of poppy isolated by Mr. Leake. The field was divided into 14 equal plots. Seven of these were lanced in the ordinary country way and the remaining seven in the Turkish fashion. In each case, however, we made subsequent lancements as long as the capsules yielded latex. The total dry opium at each lancing was estimated and also the morphine content of the dry opium of each lancing. The results are summarized below.

*Turkish versus Indian method of lancing. Yield of opium.*

TURKISH METHOD OF LANCING. GRM. OF DRY OPIUM IN EACH SUCCESSIVE LANCING							INDIAN METHOD OF LANCING. GRM. OF DRY OPIUM IN EACH SUCCESSIVE LANCING						
Plot No.	1st	2nd	3rd	4th	5th	Total	Plot No.	1st	2nd	3rd	4th	5th	Total
1 ..	15.5	27.0	18.6	10.0	3.7	74.8	2	22.2	21.4	19.8	19.2	8.9	91.5
3 ..	13.9	34.8	21.4	11.2	2.5	83.8	4	18.5	32.7	17.5	10.7	5.3	84.7
6 ..	9.9	24.9	14.9	9.3	2.0	61.0	5	12.8	23.5	13.8	10.0	3.1	63.2
8 ..	6.9	24.1	22.0	13.9	5.7	72.6	7	9.0	18.9	27.6	31.9	11.1	98.5
9 ..	16.1	25.4	18.3	5.0	2.8	67.6	10	19.9	22.3	24.3	27.7	7.3	101.5
11 ..	18.1	23.1	13.4	9.1	1.4	65.1	12	19.2	23.2	11.9	11.2	4.0	69.5
14 ..	20.8	23.5	14.8	10.0	3.5	72.6	13	15.1	23.2	14.4	13.5	2.9	69.1
TOTAL	101.2	182.8	123.4	68.5	21.6	497.5	..	116.7	165.2	129.3	124.2	42.6	578.0

*Turkish versus Indian method of lancing. % of morphine in dry opium.*

Plot No.	TURKISH METHOD OF LANCING. % OF MORPHINE IN DRY OPIUM					Plot No.	INDIAN METHOD OF LANCING. % OF MORPHINE IN DRY OPIUM				
	1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th	5th
1 ..	12.3	10.5	8.2	3.9	..	2	11.6	10.9	11.9	7.4	6.7
3 ..	12.4	10.5	6.3	2.7	..	4	12.6	10.6	8.9	4.2	4.3
6 ..	12.1	10.0	8.1	5.1	..	5	11.5	10.5	8.3	5.9	..
8 ..	12.1	11.9	10.3	5.6	..	7	14.3	11.5	11.5	8.5	5.7
9 ..	11.5	10.5	8.0	4.3	..	10	13.1	10.9	10.4	6.4	5.2
11 ..	13.5	12.2	7.5	4.2	..	12	13.1	10.5	7.6	5.0	4.8
14 ..	14.7	12.5	8.1	3.0	..	13	11.9	11.3	8.4	5.2	..
AVERAGE ..	12.6	11.3	8.1	4.1	..	..	12.6	10.8	9.6	6.1	5.5

These experiments are in favour of the Indian method of lancing. Even the out-turn of the first lancing was greater in the case of the Indian method of lancing than in the case of the Turkish method, which is rather surprising.

Unfortunately the number of capsules lanced was not counted in this experiment. This was probably however more or less the same in each plot and as there were seven plots to test each method of lancing it is unlikely that the poor results from the Turkish method of lancing were due to a smaller number of capsules being lanced. The Turkish method is much more tedious than the Indian and takes much more time both during lancing and collection.

The morphine content of the opium appears not to have been influenced by the different method of lancing.

## 2. COMPARISON OF THE USE OF TRANSVERSE INCISIONS WITH THE ORDINARY INDIAN METHOD OF VERTICAL INCISIONS.

Two groups each of 2,000 terminal capsules at the correct stage for lancing were selected. One of these groups was lanced in the usual way, *i.e.*, with a four-bladed knife used once vertically. The remaining group was lanced with a similar knife by making a transverse cut round the middle of the capsule about equal in length to the normal vertical incision. Successive lancements were made in each case at 3 days' intervals until the capsules ceased to yield latex.

*The effect of transverse versus vertical incisions on the yield and morphine content of the latex, 1918-19.*

No. of lancing	Date of lancing	VERTICAL INCISIONS		TRANSVERSE INCISIONS	
		Yield of dry opium per 1,000 capsules gm.	Percentage of morphine in dry opium	Yield of dry opium per 1,000 capsules gm.	Percentage of morphine in dry opium
1st ..	24-3-19	55.01	11.0	41.21	12.1
2nd ..	27-3-19	33.47	7.8	30.94	8.2
3rd ..	30-3-19	8.63	4.0	9.86	5.0
4th ..	2-4-19	2.15	0.0	2.50	3.0
5th ..	5-4-19	0.10	....	0.36	....
TOTAL ..	..	99.36	....	84.87	....

There is thus no marked difference in the results obtained by the two methods of lancing and if anything the Indian method has given slightly the better yield of latex.

### 3. COMPARISON OF THE INDIAN METHOD OF LANCING WITH A MODIFIED METHOD IN WHICH TWO OR MORE VERTICAL CUTS WERE MADE AT EACH LANCING.

We were led to investigate this matter since we had shown that the opium of the first lancing from each capsule is the richest in morphine and it occurred to us that by increasing the number of vertical cuts at the first lancing we might proportionately increase the yield of high morphine content opium.

In Part III above we have shown that the opium obtained from the first lancing is richest in morphine, the morphine content of the opium from the successive lancements falling away rapidly until at the fifth lancing it frequently contains only 1 to 2 per cent of morphine or even none at all as measured by the process of the *British Pharmacopœia*. The solution of the problem of the production of medical opium in India is therefore in the first place the separation of the product of the first lancing in each field. As a result of this work just referred to, it occurred to us that it might be possible to get

more opium by making two, three or more vertical cuts at each lancing instead of one as is the cultivator's usual custom. We therefore designed an experiment at Cawnpore in which a field was divided into three parts I, II and III. On part I the heads received one cut at each lancing exactly in the usual Indian fashion. On part II the heads received two cuts at each lancing and in part III the heads received three cuts vertically at each lancing. All heads lanced were counted. We were interested to see if larger yields of opium were obtained from III than from II and again whether the yields from II were greater than from I. This might be expected since a much greater length of tissue was cut. Then again if larger yields of opium were obtained by doubling or trebling the cut surface it was of importance to see whether the morphine content fell off in sympathy with the increased yield.

The first lancing took place on 10th April, 1917. On the 13th April, 1917, a second lancing under each system was carried out. After that, of course, the plants in part I had had two cuts, those in part II four cuts and that in part III six cuts. The above experiments were carried out too late in the season to give reliable results and moreover a purple-flowered Persian variety only was available by that time and it gave very poor yields. The results obtained however are given below. Fortunately Mr. Gill had a fairly large area of poppy growing at Sitoli near Almora. The poppy grown was a pure race of Katela selected by Mr. Leake. These poppies came in late enough to enable us to use them for some important experiments including a good test of the point at present being considered.

Two separate experiments were carried out there. In each case the scheme was as already described in the experiment at Cawnpore. That is, a field was divided into three portions and the three systems of lancing carried out, one on each portion. All heads lanced were carefully counted. All opium was carefully collected, weighed and analysed for morphine content by the *B. P.* 1914 method. In Experiment No. 1 five lancements were carried out except in the case where three cuts were made at each lancing. In this case the plants would not bear more than four lancements. This can be readily understood when it is realized that four lancements in this plot meant 12 vertical cuts on each capsule.

The second experiment was started later and gave only three lancements under each of the three systems. Heavy rain interfered at this point and caused the abandonment of the work.

The tables summarize the results of the two experiments at Almora and of the late one at Cawnpore. For the sake of comparison yields are

expressed on the unit of 1,000 capsules. Each plot used contained from 1,000 to 2,000 capsules.

## ALMORA EXPERIMENT No. 1. 1916-1917.

	No. and date of incision	1st 18-4-17	2nd 22-4-17	3rd 25-4-17	4th 28-4-17	5th 1-5-17	Total
<i>Plot I.</i>							
One vertical cut at each lancing, i.e., Indian method ..	Weight of dried opium per 1,000 capsules in grm. ..	21.06	18.01	18.47	13.25	17.83	88.62
	% of morphine in opium ..	11.50	8.57	5.32	3.47	1.50	..
	Grm. morphine in opium per 1,000 capsules ..	2.42	1.54	1.05	0.46	0.27	5.74
<i>Plot II.</i>							
Two vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm. ..	31.79	22.39	13.44	9.59	5.87	83.08
	% of morphine in opium ..	13.87	8.88	5.21	2.73	1.70	..
	Grm. morphine in opium per 1,000 capsules ..	4.41	1.99	0.70	0.26	0.10	7.46
<i>Plot III.</i>							
Three vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm. ..	59.82	26.67	8.78	4.08	..	99.35
	% of morphine in opium ..	10.94	5.30	2.07	1.92	..	..
	Grm. morphine in opium per 1,000 capsules ..	6.54	1.41	0.18	0.08	..	8.21

## ALMORA EXPERIMENT No. 2. 1916-1917.

	No. and date of incision	1st 25-4-17	2nd 26-4-17	3rd 1-5-17	Total
<i>Plot I.</i>					
One vertical cut at each lancing, i.e., Indian method ..	Weight of dried opium per 1,000 capsules in grm. .. ..	16.52	11.70	10.79	39.01
	% of morphine in opium .. ..	13.62	11.68	8.40	..
	Grm. morphine in opium per 1,000 capsules ..	2.25	1.37	0.91	4.53
<i>Plot II.</i>					
Two vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm. ..	21.76	11.50	8.66	41.92
	% of morphine in opium .. ..	11.99	9.00	4.96	..
	Grm. morphine in opium per 1,000 capsules ..	2.61	1.01	0.33	3.95
<i>Plot III.</i>					
Three vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm. ..	29.12	11.18	6.76	47.06
	% of morphine in opium .. ..	13.19	6.81	2.33	..
	Grm. morphine in opium per 1,000 capsules ..	3.84	0.76	0.16	4.76



## CAWNPORE (PERSIAN PURPLE VARIETY). 1916-1917.

	No. and date of incision	1st 10-4-17	2nd 13-4-17	Total
<i>Plot I.</i>				
One vertical cut at each lancing, i.e., Indian method ..	Weight of dried opium per 1,000 capsules in grm.	2.80	1.06	3.86
	% of morphine in opium .. ..	11.62	..	..
	Grm. morphine in opium per 1,000 capsules	0.33	..	..
<i>Plot II.</i>				
Two vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm.	3.50	2.03	5.53
	% of morphine in opium .. ..	12.08	..	..
	Grm. morphine in opium per 1,000 capsules	0.42	..	..
<i>Plot III.</i>				
Three vertical cuts at each lancing	Weight of dried opium per 1,000 capsules in grm.	6.53	0.24	6.77
	% of morphine in opium .. ..	12.32	..	..
	Grm. morphine in opium per 1,000 capsules	0.81	..	..

These experiments were therefore very promising, but before recommendations could be made on a large scale it was necessary that they should be tested thoroughly in the following season. Arrangements were made to do this both on our own experimental fields and also on numerous cultivators' fields throughout the Province. When subjected to this larger test however our first year's results were contradicted.

*Cawnpore experiments in 1917-18.*

A field was taken which measured 0.66 acre. It was divided into six equal parts. Plots 1 and 4 were lanced in the ordinary way and plots 2 and 5 by applying three cuts at each lancing and plots 3 and 6 by applying as many cuts at the first lancing as could conveniently be got on the surface of the capsule. In general by the latter method 5 or 6 cuts were applied per capsule and of course no second lancing was made.

In this experiment we aimed at taking about 10,000 capsules in each plot for the first lancing. At the subsequent lancings no new capsules were included. The tables show the actual number of heads taken. Moreover we have inserted in the table the figures showing the number of capsules which actually yielded latex at each lancing, for it was found that many capsules which received three incisions at the first lancing rapidly dried up and refused to yield any more latex at the later lancings.

The results are set out in the table on p. 35a opposite.

The results show that yield of opium is not proportionately increased by increasing the number of incisions at each lancing to five or six. It actually becomes less than if only one incision be made. When three incisions were made at each lancing the yield of opium at the first lancing was increased but not proportionately so. Thus with one incision the first lancing gave in the duplicate plots 35.5 and 25.9 or an average of 30.7 grm. of dry opium per 1,000 capsules.

With three incisions the first lancing gave in the duplicate plots 33.1 and 48.7 or an average of 40.9 grm. of dry opium per 1,000 capsules, an increase of 33% only. With five or more incisions at each lancing the duplicate plots gave only 16.6 and 25.3 or an average of 21.0 grm. dry opium per 1,000 capsules.

As regards the total out-turn of opium at all lancements however the average out-turn of the duplicate plots works out identically the same *viz.*, 51 grm. of dry opium per 1,000 capsules for the single and treble cut method of lancing and to 21.0 grm. of dry opium per 1,000 capsules where each capsule received one lancing of five or more cuts. Where three incisions are applied at each lancing the capsules dry up and cease to yield latex much earlier than when only one incision is made at each lancing. Thus at the time of third lancing the majority of the capsules yielded latex by the latter method, but in the case of the former method only one-fourth to one-third of them yielded latex. In this connection one may refer to Part III above (page 21) where it was indicated that if the number of incisions per lancing be increased then a longer period of time between the lancements is required by the plant in order to recover its latex-yielding power.

The effect of increasing the number of incisions per lancing on the morphine content of opium will be considered later on in the paper. At Cawnpore in 1917-18 we had two fields 44 and 50, each 0.5 acre in area, on each of which we had ten plots manured in different ways. Seed of the same race of plant was grown in each field and the plots on field 44 were exact duplicates of those on field 50 in the manner of manurial treatment, that is plot 1 on field 44 is strictly comparable with plot 1 on field 50, plot 2 with plot 2 and so on. The soil of the two fields seemed identical and cultural treatment and irrigation were carried out in exactly the same manner in the two fields. In harvesting the opium however the capsules on field 44 were lanced with a single incision at each lancing and those on field 50 with a treble incision at each lancing.

The next table gives the number of capsules lanced at the first lancing, the yield of opium, and the morphine content of the same on each plot of each field.

*One vertical incision versus three vertical incisions at each lancing.*

FIELD 44					FIELD 50				
ONE INCISION (THE INDIAN METHOD) AT EACH LANCING, 1ST LANCING ONLY					THREE INCISIONS AT EACH LANCING, 1ST LANCING ONLY				
No. of plot	No. of capsules lanced	Total grm. dry opium	Grm. dry opium per 1,000 capsules	% of morphine in dry opium	No. of plot	No. of capsules lanced	Total grm. dry opium	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium
1 ..	2736	91.4	33.4	15.0	1	1941	93.9	48.4	14.4
2 ..	3240	180.4	55.7	14.9	2	2343	122.7	52.4	15.7
3 ..	3530	164.5	46.6	15.0	3	3230	146.4	45.4	14.7
4 ..	3276	132.9	40.6	14.3	4	4044	188.9	46.7	13.7
5 ..	3180	78.5	24.7	15.1	5	2545	75.1	29.5	14.8
6 ..	4044	169.7	42.0	15.3	6	4090	182.2	44.5	12.0
7 ..	3241	70.7	31.5	15.8	7	2910	53.4	18.3	13.9
8 ..	2698	72.1	26.7	15.1	8	3812	88.0	23.1	12.6
9 ..	1910	67.7	35.4	14.7	9	2732	29.1	48.4	13.4
10 ..	3212	123.7	38.5	15.0	10	3470	198.5	57.2	16.4
Average	..	..	..	15.0	Average	..	..	..	14.2

The following table gives the yields of dry opium at each successive lancing and also the total yield per plot in grm. in the same experiment.

*Single vertical incision at each lancing versus three vertical incisions.**Yield of dry opium in grm. per plot.*

FIELD 44								FIELD 50							
SINGLE INCISION AT EACH LANCING								THREE INCISIONS AT EACH LANCING							
Plot No.	Number of lancing							Plot No.	Number of lancing						
	1st	2nd	3rd	4th	5th	6th	Total		1st	2nd	3rd	4th	5th	Total	
1	91.4	85.1	24.7	18.8	10.5	4.0	234.5	1	93.9	37.0	32.5	2.2	9.1	174.7	
2	180.4	157.0	53.9	54.4	30.3	13.5	489.5	2	122.7	91.5	68.4	2.5	2.2	287.3	
3	164.5	124.1	89.5	33.9	5.2	3.2	420.4	3	146.4	74.8	9.1	..	..	230.3	
4	132.9	120.3	68.6	29.7	11.8	2.7	371.4	4	188.9	45.2	13.3	..	..	247.4	
5	78.5	66.2	24.1	13.8	7.4	1.6	191.6	5	75.1	29.4	5.3	..	..	109.8	
6	169.7	134.9	39.1	12.4	6.8	1.7	364.6	6	182.2	34.7	8.8	..	..	225.7	
7	70.7	83.1	52.7	10.7	11.7	3.7	232.3	7	53.4	20.3	6.0	..	..	79.7	
8	72.1	67.8	35.7	12.5	9.0	2.2	193.3	8	88.0	17.6	3.5	..	..	109.1	
9	67.7	50.4	32.7	24.6	12.0	6.4	193.8	9	79.6	34.7	5.1	..	..	119.4	
10	123.7	88.9	40.3	22.2	9.8	3.2	288.1	10	198.5	49.1	44.3	2.2	4.2	298.3	

Hence in every case, except plot 10, as regards the total out-turn of opium one incision at each lancing has given a bigger out-turn than three incisions. In this particular experiment even the yield per 1,000 capsules at the first lancing has in only a few cases been greater with the latter method of lancing. On the whole also the morphine content of opium of the first lancing tends to be lower in the plots in which three incisions were made per lancing than in the others.

As a laboratory check on the field experiments we had a number of poppy plants grown in pots, each pot containing one plant. Eighty of these plants were selected having only one capsule per plant and care was taken to select 80 plants as nearly as possible alike in growth.

These plants were then arranged in 8 groups, each of 10 plants. The plants in two of these groups were then lanced in the ordinary Indian fashion with a knife having four blades only, one incision being made at each lancing. A second set of two groups was taken in which two incisions were made at each lancing. In the third set of two groups three incisions were made at each lancing and in the remaining set of two groups six incisions were made. The lancements were carried out in the afternoon at the same time on the same day in each case, after the capsule had been carefully cleaned with water and alcohol till free from wax. Next morning the latex was carefully washed off with distilled water into a weighed dish, the produce of each set of ten plants being kept separately. Thus there were duplicate determinations for each method of lancing. The water then was carefully evaporated and the residues dried to constant weight in the water bath. The same procedure was followed with the produce of a subsequent lancing which took place two days later.

The following results were obtained:—

*Comparative influence of single, double, treble and six vertical cuts at each incision on yield of opium by plants grown in pots.*

No. of incisions at each lancing	No. of plants lanced	Do. yielding latex at 1st lancing	Do. yielding latex at 2nd lancing	YIELD OF DRY OPIUM IN GRM.					
				1ST LANCING		2ND LANCING		Total opium per plant	Average of duplicates
				Opium per plant	Average of duplicates	Opium per plant	Average of duplicates		
1	10	10	10	0.0241	0.0294	0.0298	0.0292	0.0539	0.0586
1	10	10	9	0.0347		0.0284		0.0631	
2	10	10	8	0.0304	0.0352	0.0158	0.0189	0.0462	0.0541
2	10	10	9	0.0399		0.0220		0.0619	
3	10	10	6	0.0377	0.0374	0.0102	0.0114	0.0479	0.0488
3	10	10	7	0.0370		0.0135		0.0495	
6	10	10	1	0.0279	0.0277	0.0023	0.0011	0.0302	0.0288
6	10	10	0	0.0275		0.0000		0.0275	

These results confirm those of the field experiments in showing that there is nothing to be gained in the long run by increasing the number of incisions at each lancing. When six incisions are made into the capsule at the same time the amount of latex exuded is decreased much below that obtained by making a single incision. This result is of great interest.

*District experiments.*

Through the courtesy of the then officiating Opium Agent, Mr. J. R. Pearson, C.I.E., I.C.S., the District Opium Officials were asked to co-operate with us in carrying out such experiments as they were able to find time for. The District Opium Officers are busy officials and all were of course not able to give the necessary time for this work. We have however to thank Messrs. Mawson, Godfrey, James, Rose, Browne, Farnon, Hill, Dawe, G. O'B. Power, G. A. Levett Yeats, C.I.E., I.S.O., D. G. Harris, W. Harris, Cartland and Emerson for the large amount of trouble they took to help us in this work. In each case a field of poppy of, say, an area of one *bigha* (5-8ths of an acre) was selected, as uniform as possible in growth. This was divided into 2, 4, or 6 equal plots, though owing to local circumstances modifications of the method of division of the field had to be made as will be seen later. On half the plots the cultivator carried out his ordinary method of lancing and in the remaining plots three vertical incisions were made at each lancing. In most cases the total produce of each successive lancing was sent to us. In all cases except in that of the experiment at the Ghazipur Factory, samples were sent to us for analysis. All the necessary analyses and weighments for the Ghazipur Factory experiment were carried out at the Factory. The results are summarized in the following table:—



*Indian method of lancing versus three incisions at each lancing. District Officers' experiments. Yields of dry opium in lb. per acre at each lancing.*

Sub-Agency	ONE INCISION AT EACH LANCING (INDIAN METHOD)							THREE INCISIONS AT EACH LANCING						
	Plot No.	First	Second	Third	Fourth	Fifth	Total	Plot No.	First	Second	Third	Fourth	Fifth	Total
Bara Banki	D	lb. oz. 17 1	lb. oz. 7 1	lb. oz. ..	lb. oz. ..	lb. oz. ..	lb. oz. 24 8	A	lb. oz. 19 0	lb. oz. 4 10	lb. oz. ..	lb. oz. ..	lb. oz. ..	lb. oz. 23 10
	E	13 0	7 6	..	..	..	20 6	B	16 6	3 2	..	..	..	19 8
	F	8 7	5 12	..	..	..	14 3	C	10 2	3 9	..	..	..	13 11
	Aver.	12 13	6 12	..	..	..	19 11	Aver.	15 3	3 12	..	..	..	18 15
Budaun (Ujhiani) ..	A	3 10	..	11 12	..	..	15 6	B	6 5	..	7 5	..	..	13 10
Budaun (Salempur).	I A	4 6	..	11 10	..	..	16 0	I C	5 6	..	6 10	..	..	12 0
	I B	4 6	..	16 1	..	..	20 7	I D	5 6	..	6 10	..	..	12 0
	Aver.	4 6	..	..	..	..	18 3	Aver.	5 6	..	6 10	..	..	12 0
	II A	6 4	..	14 5	..	..	20 9	II C	9 0	..	7 9	..	..	16 9
Ditto.	II B	3 0	..	13 8	..	..	16 8	II D	5 6	..	7 11	..	..	13 1
	Aver.	4 10	..	..	..	..	18 8	Aver.	7 3	..	..	..	..	14 13
	A	16 5	9 6	5 3	2 15	1 8	35 5	B	10 0	4 5	2 0	1 2	0 8	17 15
	C	10 10	5 3	3 0	1 8	0 11	21 0	D	14 6	5 3	2 5	0 13	0 8	23 3
Balia ..	Aver.	13 7	7 4	4 1	2 3	1 1	28 2	Aver.	12 3	4 12	2 3	0 15	0 8	20 9
Ankin (Cawnpore) ..	I	5 4	7 3	5 12	4 0	0 13	23 0	II	9 2	12 7	5 12	2 0	0 6	29 11
	III	6 9	12 0	8 10	3 0	1 5	31 8	IV	8 14	8 14	3 2	1 4	0 6	22 8
	Aver.	5 14	..	..	..	..	27 4	Aver.	9 0	..	..	..	..	26 1
	I I	4 4	4 15	1 13	0 10	0 8	12 2	I 2	6 7	2 8	0 8	0 2	0 5	9 14
Rae Bareilly (Dalmau)	I 3	6 7	4 13	1 12	0 9	0 10	14 3	I 4	6 9	2 0	0 9	0 3	0 4	9 9
	Aver.	5 5	..	..	..	..	13 2	Aver.	6 8	..	..	..	..	9 11
	II I	4 6	8 4	7 2	3 14	0 14	24 8	II 2	5 7	11 8	5 5	1 13	0 12	24 13
	II 3	3 13	5 12	6 9	4 5	1 10	23 0	II 4	7 10	2 5	5 13	1 14	0 8	23 12
Ditto.	Aver.	4 1	..	..	..	..	23 12	Aver.	5 5	..	..	..	..	24 4

Sub-Agency	ONE INCISION AT EACH LANCING (INDIAN METHOD)						THREE INCISIONS AT EACH LANCING							
	Plot No.	First	Second	Third	Fourth	Fifth	Total	Plot No.	First	Second	Third	Fourth	Fifth	Total
{ Fyzabad	11 A	9	3	11	4	3	24	14 A	12	5	1	9	..	22
	12 A	14	1	5	10	0	21	15 A	16	0	8	0	..	24
	13 A	9	3	5	5	13	15	16 A	12	0	3	2	0	15
	Aver.	10	13	..	..	..	20	Aver.	13	7	..	..	..	20
{ Ditto.	111 A	15	2	10	15	1	26	114 A	13	3	7	9	..	20
	112 A	19	2	8	14	0	28	115 A	8	14	10	..	..	23
	113 A	17	9	6	0	..	23	116 A	13	0	2	8	..	15
	Aver.	17	4	..	..	..	25	Aver.	11	11	..	..	..	19
{ Ghazipur	A	8	1	3	14	3	17	B	5	8	3	14	0	11
	D	7	12	3	10	4	16	C	6	6	2	7	0	10
	Aver.	7	14	..	..	..	17	Aver.	5	15	..	..	..	11
	XYZ	1	11	3	10	3	12	ABC	2	11	4	2	1	12
{ Lucknow	A	10	7	7	14	..	18	a	9	1	3	4	..	12
	B	7	4	8	12	..	16	b	10	4	3	2	..	13
	D	12	3	8	15	..	21	d	13	1	3	2	..	16
	Aver.	9	15	8	9	..	18	Aver.	10	13	..	..	..	13
{ Moradabad	A	0	3	0	8	1	11	B	1	2	1	14	1	9
							6							0
{ Rae Bareli	A	..	..	..	..	..	..	B	..	..	..	..	..	..
	C	..	..	..	..	..	..	D	..	..	..	..	..	..
	Aver.	..	..	..	..	..	..	Aver.	..	..	..	..	..	..

\* Includes out-turn of four subsequent lancements.

† Includes out-turn of two subsequent lancements.

*Indian method of lancing versus three incisions at each lancing. Morphine content of opium dried at 100° C. District Officers' experiments.*

Sub-Agency	ONE INCISION AT EACH LANCING (INDIAN METHOD)				THREE INCISIONS AT EACH LANCING			
	Plot No.	NUMBER OF LANCING			Plot No.	NUMBER OF LANCING		
		First	Second	Third		First	Second	Third
Bara Banki ..	D	12.2	..	..	A	11.3	..	..
	E	11.7	..	..	B	10.5	..	..
	F	10.6	..	..	C	11.5	..	..
	Aver.	11.5	..	..	Aver.	11.1	..	..
Budaun (Ujhiani) ..	A	13.5	..	..	B	13.0	..	..
Budaun (Salempur) ..	A I	14.3	..	..	C I	12.4	..	..
	B I	14.4	..	..	D I	13.8	..	..
	Aver.	14.3	..	..	Aver.	13.1	..	..
Ditto ..	A II	16.5	..	..	C II	14.4	..	..
	B II	18.0	..	..	D II	16.8	..	..
	Aver.	17.2	..	..	Aver.	15.6	..	..
Ballia ..	A	11.6	8.3	5.9	B	12.0	6.7	5.0
	C	11.9	7.7	6.6	D	11.4	6.8	6.2
	Aver.	11.7	8.0	6.2	Aver.	11.7	6.7	5.6
Ankin (Cawnpore) ..	I	15.7	12.5	9.5	II	14.0	12.8	11.8
	III	16.7	13.7	10.3	IV	13.9	13.2	11.1
	Aver.	16.2	13.1	9.9	Aver.	13.9	13.0	11.4
Rae Bareli (Dalmau) ..	I 1	10.3	5.7	4.4	I 2	8.3	5.7	4.6
	I 3	9.7	5.5	4.1	I 4	8.5	6.6	4.8
	Aver.	10.0	5.6	4.2	Aver.	8.4	6.1	4.7
Ditto ..	II 1	12.4	10.6	8.6	II 2	11.3	10.0	7.7
	II 3	11.2	11.7	9.6	II 4	11.1	11.0	6.7
	Aver.	11.8	11.1	9.1	Aver.	11.2	10.5	7.2
Fyzabad ..	I 1A	13.1	9.4	5.5	I 1	11.8	8.6	7.0
	I 2A	13.9	9.4	6.9	I 2	12.0	8.0	6.0
	I 3A	13.6	8.5	6.5	I 3	13.3	7.3	7.0
	Aver.	13.5	9.1	6.3	Aver.	12.4	8.0	6.7
Ditto ..	II 1A	14.7	9.8	..	II 1	12.6	11.0	..
	II 2A	13.7	7.5	..	II 2	12.5	8.1	..
	II 3A	12.3	9.2	..	II 3	11.8	9.6	..
	Aver.	13.6	8.8	..	Aver.	12.3	9.6	..

Sub-Agency	ONE INCISION AT EACH LANCING ( INDIAN METHOD)				THREE INCISIONS AT EACH LANCING			
	Plot No.	NUMBER OF LANCINGS			Plot No.	NUMBER OF LANCINGS		
		First	Second	Third		First	Second	Third
Ghazipur..	A	11.3	8.1	9.2	B	12.3	..	8.3
	D	11.9	9.7	8.1	C	11.2	..	8.3
	Aver.	11.6	8.9	8.6	Aver.	11.7	..	8.3
Ghazipur Factory ..	X	12.8	13.2	13.0	A	13.6	13.5	12.9
	Y	13.1	13.4	12.8	B	13.6	13.8	12.6
	Z	13.4	13.6	12.3	C	13.8	13.2	13.5
	Aver.	13.1	13.4	12.7	Aver.	13.7	13.5	13.0
Lucknow ..	A	11.3	7.9	38.1	a	10.1	7.9	9.0
	B	11.8	8.0	..	b	11.3	6.8	..
	D	12.6	7.6	..	d	7.3	6.6	..
	Aver.	11.9	7.9	..	Aver.	9.6	7.1	..
Moradabad ..	A	18.0	15.9	15.8	B	15.8	14.9	12.5
Rae Bareli ..	A	13.8	10.0	7.1	B	11.3	11.7	8.4
	C	12.8	9.4	7.7	D	11.4	10.2	8.8
	Aver.	13.3	9.7	7.4	Aver.	11.3	10.9	8.6

A study of the above tables shows that, as regards the total out-turn of opium per acre, the ordinary Indian method of applying one vertical cut at each lancing has given a greater out-turn in almost every case than that of three incisions at each lancing. In some cases the difference in favour of the Indian method is considerable.

As regards the yield of opium at the first lancing however the method of making three incisions at each lancing has usually given the larger yield but the increase is not proportional to the number of incisions made.

The morphine content of opium produced at the first lancing strongly indicates that, when three incisions are made at each lancing, the morphine content of the opium of the first lancing is distinctly lower than when only one incision is made at each lancing. The following table compiled from the two preceding tables sets out side by side the average figures for yield of opium and its morphine content of the first lancing in the case of both methods of lancing.

					SINGLE LANCING		TREBLE LANCING	
					Yield	Morphine content	Yield	Morphine content
					lb. oz.		lb. oz.	
Bara Banki	..	..	..	..	12 15	11.5	15 3	11.1
Budaun (Ujhiani)	..	..	..	..	3 10	13.5	6 5	13.0
Budaun (Salempur)—A	..	..	..	..	4 6	14.3	5 6	13.1
Ditto	B	..	..	..	4 10	17.2	7 3	15.6
Ballia	..	..	..	..	13 7	11.7	12 3	11.7
Ankin	..	..	..	..	5 14	16.2	9 0	13.9
Rae Bareli (Dalmau)—A	..	..	..	..	5 5	10.0	6 8	8.4
Ditto	B	..	..	..	4 1	11.8	5 7	11.2
Fyzabad A	..	..	..	..	10 13	13.5	13 7	12.4
Ditto B	..	..	..	..	17 4	13.6	11 11	12.3
Ghazipur	..	..	..	..	7 14	11.6	5 15	11.7
Ditto Factory	..	..	..	..	1 11	13.1	2 11	13.7
Lucknow	..	..	..	..	9 15	11.9	10 13	9.6
Moradabad	..	..	..	..	0 3	18.0	1 2	15.8
Rae Bareli	..	..	..	..	..	13.3	..	11.3

It will be seen that in certain cases the method of triple incisions has produced opium of considerably lower morphine content than that on the check plots where the ordinary method of lancing was carried out and that in those cases the yield of opium was distinctly higher in the method of treble lancing.

The experiments at Rae Bareli, Budaun (Salempur)—B, and Ankin are good illustrations of this.

In order to test the point properly however one would have to lance a number of capsules in the ordinary way and estimate the yield of opium and its morphine content. The same number of similar capsules would then have to be lanced by the method of treble incisions and the yield and morphine content again estimated. We have made a certain number of experiments on these lines, but they were inconclusive, because the two methods of lancing gave more or less the same yield of opium per capsule.

In the case of the Budaun (Salempur) and Ankin experiments we have however data for the yield of opium per 1,000 capsules by each system of lancing. These figures and the morphine content of the opium obtained are set out here side by side.



*Opium of first lancing.*

District	No. of plot	INDIAN METHOD OF LANCING				TREBLE INCISIONS AT EACH LANCING				
		No. of capsules lanced	Total dry opium gm.	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium	No. of plot	No. of capsules lanced	Total dry opium gm.	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium
Budaun (Salempur)	I (A)	4841	322	67.5	14.3	I (C)	4689	374	79.7	12.4
	I (B)	3758	322	85.6	14.4	I (D)	5665	374	65.8	13.8
	II (A)	3656	623	110.4	16.3	II (C)	8246	849	136.0	14.4
	II (B)	5843	283	48.4	18.0	II (D)	7501	589	67.9	16.8
Ankin	I	3408	235	69.0	15.7	II	4589	424	92.4	14.0
	III	4044	321.9	79.6	16.7	IV	4013	395.9	98.7	13.9

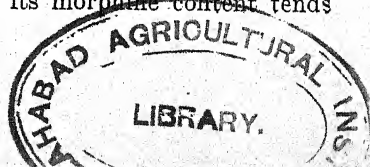
In five out of six of these pairs of plots it will be seen that the yield of opium per 1,000 capsules at the first lancing was distinctly in favour of the method of making three incisions at each lancing. In all these five cases moreover the percentage of morphine in the opium was much lower than that in the opium from the plots in which only one incision was made at each lancing, the difference being from 1.2 to 2.8 per cent morphine. In the sixth pair of plots [Budaun I (B) and (D)] the method of making treble incisions at each lancing gave a lower yield per 1,000 capsules than the duplicate plot receiving only one incision per lancing and in this case there was only a difference in morphine content of 0.6 per cent. between the two samples of opium.

In conclusion we would say that it is inadvisable to encourage the cultivators to make three vertical cuts at each lancing, since the total yield of opium is not increased but often decreased.

Moreover the morphine content of the opium produced at the first lancing from capsules receiving three incisions at each lancing tends to be lower than that of opium produced by the ordinary method of lancing in which only one incision was made at each lancing.

#### 4. COMPARISON OF INDIAN METHOD OF LANCING OF ONE FULL LENGTH INCISION AT EACH LANCING WITH A MODIFICATION OF THE METHOD IN WHICH THE LENGTH OF THE INCISION WAS MUCH REDUCED.

The experiment just described indicates that, if a large amount of opium be removed from the capsule at the first lancing, its morphine content tends



to be lower than if a smaller amount had been removed. Moreover it is our experience that the morphine content of the opium of the second and subsequent lancements falls off much more rapidly when three incisions are made at each lancing than when only one incision is made. For good illustrations of this see the figures on pages 34 and 35A. These are again set out here for convenience in the following table :—

*Table illustrating the more rapid fall in morphine content of the opium at each successive lancing when three incisions are made per lancing as compared with the method of making one incision at each lancing.*

		ONE VERTICAL INCISION AT EACH LANCING					THREE VERTICAL INCISIONS AT EACH LANCING.				
		Plot No.	NO. OF LANCING				Plot No.	NO. OF LANCING			
			1st	2nd	3rd	4th		1st	2nd	3rd	4th
Cawnpore 1917-18 (from table).	% morphine ..	1	12.5	10.4	7.0	..	2	14.1	8.7	6.1	..
	Yield of dry opium per 1,000 capsules ..	1	35.5	12.4	5.4	..	2	33.1	11.5	2.4	..
Do.	% morphine ..	4	13.8	10.1	7.0	..	5	13.3	7.8	6.0	..
	Yield of dry opium per 1,000 capsules ..	4	25.9	12.5	5.1	..	5	48.7	4.9	0.9	..
Almora (A) 1916-17	% morphine ..	1	11.5	8.6	5.7	3.5	III	10.9	5.3	2.1	1.9
	Yield of dry opium per 1,000 capsules ..	1	21.1	18.0	18.5	13.2	III	59.8	26.7	8.8	4.1
Do. (B)	% morphine ..	1	13.6	11.7	8.4	..	III	13.2	6.8	2.3	..
	Yield of dry opium per 1,000 capsules ..	1	16.5	11.7	10.8	..	III	29.1	11.2	6.8	..

The yield of opium per 1,000 capsules at each successive lancing has also been inserted for convenience of reference.

It will at once be seen that the morphine content of the opium from the second lancements is much lower in the case of capsules which received three incisions instead of one at each lancing. This is much more marked in the Almora experiments where the method of treble incisions at each lancing removed so much more opium per 1,000 capsules than did the method of single incisions at the first lancing.

It occurred to us to investigate the effect on the yield of opium at each successive lancing and its morphine content, of removing only a very small amount of latex at each incision.

*1917-18 experiments.*

Tests were made in the three successive seasons, 1917-18, 1918-19 and 1919-20.

The 1917-18 experiment was carried out at Douglas Dale in the Himalayas on a pure race of poppy. About 2,000 single-capsuled plants on the same plots were marked with white labels. About 750 similar plants on the same plots were marked with blue labels. The latter plants were lanced in the usual way with vertical incisions about 1" long, using a three-bladed knife. The former were lanced in a similar way but the incisions were made one-third of an inch long. Successive lancements were made, usually at two days' intervals, as long as the capsules yielded latex. The total dry matter and the morphine content of the opium of each lancing were then estimated. At each collection the opium of each series was collected in two portions, in order to provide a check on the work. All the data obtained are set out in the next table.

*Table showing effect of small versus long incisions at each lancing on the yield and morphine content of the opium at each successive lancing.*

Date of lancing	No. of lancing	NO. OF HEADS YIELDING LATEX		GRM. DRY OPIUM PER SAMPLE		GRM. DRY OPIUM PER 1,000 HEADS IN EACH SAMPLE		Grm. dry opium per 1,000 capsules average	% MORPHINE IN DRY OPIUM		% morphine in dry opium average	Grm. morphine removed per 1,000 capsules
		Sample (a)	Sample (b)	Sample (a)	Sample (b)	Sample (a)	Sample (b)		Sample (a)	Sample (b)		
4-4-18	1	362	390	13.6	11.6	37.6	29.7	33.6	11.3	12.0	11.6	3.90
7-4-18	2	362	380	10.9	10.1	30.0	25.9	27.9	8.0	6.4	7.2	2.01
10-4-18	3	351	375	10.8	8.7	29.9	22.2	26.1	4.3	2.5	3.4	0.88
13-4-18	4	330	333	15.4	9.3	42.6	24.0	33.3	1.4	1.4	1.4	0.47
16-4-18	5	220	250	4.8	3.9	13.1	10.1	11.6	1.3	0.0	0.6	0.00
19-4-18	6	167	141	4.4	..	..	..	5.9	..	..	..	..
22-4-18	7	82	57	3.0	..	..	..	4.0	..	..	..	..
25-4-18	8	7	3	..	..	..	..	..	..	..	..	..
Total	..	..	..	..	..	..	..	142.4	..	..	..	7.26

*A. Blue labels, i.e., full length incisions.*

Date of lancing	No. of lancing	No. of HEADS YIELDING LATEX		GRM. DRY OPIUM PER SAMPLE		GRM. DRY OPIUM PER 1,000 HEADS IN EACH SAMPLE		Grm. dry opium per 1,000 capsules average	% MORPHINE IN DRY OPIUM		% morphine in dry opium average	Grm. morphine removed per 1,000 capsules
		Sample (a)	Sample (b)	Sample (a)	Sample (b)	Sample (a)	Sample (b)		Sample (a)	Sample (b)		
<i>B. White labels, i.e., one-third length incision.</i>												
4-4-18	1	1,050	990	11.0	13.1	10.5	15.2	11.8	13.7	13.0	13.3	1.57
7-4-18	2	1,050	990	14.9	17.5	14.2	17.7	15.9	10.3	10.7	10.5	1.67
10-4-18	3	1,046	983	15.8	20.6	15.1	20.9	18.0	8.7	8.3	8.5	1.53
13-4-18	4	1,039	955	20.0	32.9	19.1	33.2	26.1	5.0	4.7	4.9	1.28
16-4-18	5	1,034	937	25.1	16.9	23.9	17.1	20.5	3.1	2.5	2.8	0.57
19-4-18	6	942	927	15.1	9.1	14.3	9.2	11.7	..	..	..	..
22-4-18	7	783	811	9.1	5.3	8.6	5.4	7.0	..	..	..	..
25-4-18	8	486	503	8.9	..	4.4	..	..	..	..	..	..
28-4-18	9	74	92	..	..	..	..	..	..	..	..	..
1-5-18	10	80	65	..	..	..	..	..	..	..	..	..
4-5-18	11	25	18	..	..	..	..	..	..	..	..	..
Total	..	..	..	..	..	..	..	111.8	..	..	..	6.62

In the first place the table shows that by making only small incisions at each lancing the capsule is capable of yielding latex over a considerably longer period than if full length incisions are made. Thus on 25th April, 1918, at the 8th lancing about half of the capsules receiving only small incisions were still yielding latex, whereas in the case of the capsules receiving full length incisions less than half yielded latex at the sixth incision on the 19th April, 1918. As regards the first lancing the yield of opium was roughly proportional to the length of the cut. The total out-turn of all lancements was considerably greater in the case of the capsules receiving full length incisions.

As regards the morphine content of each successive lancing there is a notable difference. The small incisions gave opium at the first lancing of distinctly greater morphine content than that from the first lancing with full length incisions and whereas in the former case the morphine content of the opium from the 2nd, 3rd and 4th lancing was 10.8, 8.5 and 4.9 per cent respectively, in the case of the full length incision it was much lower,

*viz.*, 7.2, 3.4 and 1.4 per cent respectively. It would appear therefore to be indicated that a given capsule is only capable of giving a certain amount of morphine whatever method of lancing be employed. In the last column of the table is given a figure showing the calculated amount of morphine removed at each lancing per 1,000 capsules. It will be seen that all the lancements in the case of full length incisions yielded about 7.26 gm. morphine per 1,000 capsules. With incisions only one-third of the length of the total lancements yielded about 6.63 gm. morphine per 1,000 capsules or practically the same within the limits of error in both methods of lancing.

*1918-19 experiments.*

In this season further experiments on similar lines were carried out at Cawnpore. Two groups of terminal capsules ready for lancing were selected. One group consisted of about 2,000 capsules and the remaining group had about 6,000. The former group was lanced with an incision of the normal length with a four-bladed knife. The remaining group was lanced with a similar knife, but the incisions were made about one-third the length of the incisions made in the case of the other group. Successive lancements were carried out in a similar manner in each case until the capsules yielded no more latex. An interval of three days was left between each successive lancing. The table summarizes the results.

Group	Nature of incision	Date of lancing	No. of lancing	No. of heads lanced	Grm. dry opium	Grm. dry opium per 1,000 heads	Percentage of morphine in opium dried at 100° C.	Grm. morphine removed per 1,000 capsules
I	Normal	24-3-19	1st	2000	89.63	44.81	11.8	5.29
		27-3-19	2nd	..	69.12	34.56	7.4	2.56
		30-3-19	3rd	..	23.13	11.56	4.8	0.55
		2-4-19	4th	..	3.92	1.96	2.4	0.05
		5-4-19	5th	..	0.36	0.18	....	..
			Total	..	186.16	93.08		8.45
II	One-third normal length	25-3-19	1st	6000	189.30	31.55	13.8	4.35
		28-3-19	2nd	..	175.26	29.21	11.2	3.27
		31-3-19	3rd	..	105.28	17.54	7.1	1.24
		3-4-19	4th	..	30.52	5.09	5.3	0.27
		6-4-19	5th	..	2.25	0.37	0.0	0.00
			Total	..	502.61	83.76		9.13



These results confirm those of the previous year and call for no further remarks.

*1919-20 experiments.*

The experiment was repeated at Cawnpore in the season 1919-20. Instead of comparing the latex produced from incisions of the usual length with that from incisions one-third as long, the experiment was designed to test the effect of making incisions of normal length as compared with making them only one-sixth as long. It was thought that possibly any difference might be magnified in this way. The 1919-20 experiment was duplicated, each pair of duplicates being carried out in a separate field. The table sufficiently describes the experiments together with the results :—

Field No.	Nature of incision	Date of lancing	No. of lancing	No. of heads lanced	Grm. dry opium	Grm. dry opium per 1,000 heads	Percentage of morphine in opium dried at 100° C.	Grm. morphine removed per 1,000 capsules
40	Normal .	13-3-20	1st	493	15.29	30.87	15.72	4.85
		16-3-20	2nd	..	26.98	10.29	10.29	5.63
		19-3-20	3rd	..	12.15	24.66	6.02	1.48
			Total		54.42	110.24		11.96
	One-sixth normal length	13-3-20	1st	1561	25.98	16.64	17.31	2.88
		16-3-20	2nd	..	15.19	9.73	16.03	1.56
		19-3-20	3rd	..	30.82	19.74	14.30	2.82
			Total		71.99	46.11		7.26
	Normal .	13-3-20	1st	471	22.04	46.79	16.91	7.91
		16-3-20	2nd	..	26.37	56.01	11.26	6.31
		19-3-20	3rd	..	13.50	28.67	6.37	1.82
			Total		61.91	131.47		14.04
46	One-sixth normal length	13-3-20	1st	1365	19.88	14.56	17.20	2.50
		16-3-20	2nd	..	30.15	22.09	16.55	3.66
		19-3-20	3rd	..	24.78	18.16	13.42	2.44
			Total		74.81	54.81		8.60
	Normal .	13-3-20	1st	471	22.04	46.79	16.91	7.91
		16-3-20	2nd	..	26.37	56.01	11.26	6.31

Broadly speaking the results confirm those of the previous seasons. The yield of latex is not proportional to the length of the incision however. When the incisions were only one-sixth of the normal length they yielded from one-third to half as much latex as incisions of the normal length. Using small incisions the decrease in morphine content of the opium from each successive lancing was nothing like so rapid as in the case of incisions of the normal length. It is unfortunate that the experiment was unable to be continued after the third lancing. In the case of tiny incisions the opium at the third lancing still contained 14.3 and 13.4 per cent of morphine on the two plots respectively as against 6.0 and 6.4 per cent in the case of the capsules receiving incisions of normal length. The last column in the table shows that up to this time the total morphine removed per 1,000 capsules receiving tiny incisions was still far short of that removed from the capsules receiving incisions of normal length and hence one should expect the latex to be of higher morphine concentration in the former case.

#### 5. THE EFFECT OF THE NUMBER OF KNIFE-BLADES ON THE YIELD AND MORPHINE CONTENT OF THE OPIUM.

Knives were made up containing 2, 4 and 6 blades. Experiments were carried out both at Douglas Dale in the season 1917-18 and at Cawnpore in 1919-20. The poppy used was a pure race and only single-capsuled plants were included in the experiment.

##### *Douglas Dale experiments.*

*Two-bladed knife.* The experiment was duplicated. In one 305 and in the other 307 capsules were lanced at each lancing.

*Four-bladed knife.* Only one experiment was carried out under this head, 579 capsules being employed.

*Six-bladed knife.* This experiment was also duplicated, 268 and 289 capsules respectively being lanced.

The dry matter and morphine content of the opium from each lancing were estimated. The number of capsules actually yielding latex at each lancing was also recorded in each case.

The results are summarized in the following table:—

Table showing the influence of using lancing knives containing 2, 4 and 6 blades respectively on the yield and composition of the opium from each successive lancing. Douglas Dale 1917-18.

Date of lancing	No. of lancing	Number of capsules lanced	Number of capsules yielding latex	Grm. dry opium	Grm. dry opium per 1,000 capsules	Percentage of morphine in opium	Total morphine per 1,000 capsules grm.
Two-bladed knife.							
8-4-18 ..	1st	(a) 305	305	5.94	19.5	12.0	....
		(b) 307	307	5.05	16.4	12.2	....
				Average	18.0	12.1	2.18
11-4-18 ..	2nd	(a) 305	293	7.23	23.7	7.8	....
		(b) 307	292	6.45	21.0	7.7	....
				Average	22.3	7.7	1.72
14-4-18 ..	3rd	(a) 305	291	7.72	25.3	5.6	....
		(b) 307	288	7.23	23.5	6.4	....
				Average	24.4	6.0	1.46
17-4-18 ..	4th	(a) 305	284	7.73	25.3	2.8	....
		(b) 307	272	6.93	22.6	3.5	....
				Average	24.0	3.1	0.74
20-4-18 ..	5th	(a) 305	181	2.90	9.5	1.3	....
		(b) 307	195	4.49	14.6	1.2	....
				Average	12.0	1.2	0.14
23-4-18 ..	6th	(a) 305	97	1.50	4.9	0.0	....
		(b) 307	82	1.30	4.2	0.0	....
				Average	4.5	0.0	....
28-4-18 ..	7th	(a) 305	18	0.20	0.6	0.0	....
		(b) 307	19	0.25	0.8	0.0	....
				Average	0.7	0.0	....
				Total..	106.5	....	6.24
Four-bladed knife.							
8-4-18 ..	1st	579	579	11.96	20.62	13.3	2.74
11-4-18 ..	2nd	579	570	19.58	33.76	8.9	3.00
14-4-18 ..	3rd	579	540	17.12	29.52	5.6	1.65
17-4-18 ..	4th	579	449	10.57	18.22	2.1	0.38
20-4-18 ..	5th	579	261	3.24	5.59	2.8	0.16
23-4-18 ..	6th	579	30	0.30	0.52	....	....
26-4-18 ..	7th	579	9	0.13	0.22	....	....
				Total..	108.45		7.93

Date of lancing	No. of lancing	Number of capsules lanced	Number yielding latex	Grm. dry opium	Grm. dry opium per 1,000 capsules	Percent- age of morphine in opium	Total morphine per 1,000 capsules grm.
Six-bladed knife.							
8-4-18 ..	1st	(a) 268	268	11.96	40.0	12.5	....
		(b) 289	289	11.62	40.2	12.5	....
				Average	40.1	12.5	5.01
11-4-18 ..	2nd	(a) 268	264	10.85	40.5	6.7	....
		(b) 289	287	12.51	43.3	8.5	....
				Average	41.9	7.6	3.18
14-4-18 ..	3rd	(a) 268	245	7.77	20.9	2.8	....
		(b) 289	220	7.37	25.5	3.7	....
				Average	23.2	3.2	0.74
17-4-18 ..	4th	(a) 268	180	2.23	8.3	2.6	....
		(b) 289	171	2.52	8.7	1.5	....
				Average	8.5	2.0	0.17
20-4-18 ..	5th	(a) 268	94	2.15	8.0	2.1	....
		(b) 289	107	2.56	8.7	1.7	....
				Average	8.3	1.9	0.16
23-4-18 ..	6th	(a) 268	14	0.17	0.6	0.0	....
		(b) 289	55	0.90	3.1	0.0	....
				Average	1.8	0.0	....
26-4-18 ..	7th	(a) 268	0	0.00	0.0	0.0	....
		(b) 289	8	0.11	0.4	0.0	....
				Average	0.2	0.0	....
				Total..	124.0		9.26

It will be seen that as the number of blades on the knife is increased the number of successive lancing during which a capsule can yield latex diminishes. The results bearing on this are summarized in the next table for convenience.

*Table showing the ratio of number of heads lanced to those yielding latex at each successive lancing with 2- 4- and 6-bladed knives.*

No. of lancing		No. OF BLADES IN KNIFE					
		2		4		6	
		Total No. of heads lanced	No. yielding latex	Total No. of heads lanced	No. yielding latex	Total No. of heads lanced	No. yielding latex
1	..	612	612	579	579	557	557
2	..	612	585	579	570	557	551
3	..	612	579	579	540	557	465
4	..	612	556	579	449	557	351
5	..	612	376	579	261	557	201
6	..	612	179	579	30	557	69
7	..	612	37	579	9	557	8

Although the total number of heads lanced in each case was more or less the same, it will be at once seen that the number yielding latex at each lancing diminished more rapidly with the four-bladed knife than with the two-bladed one and more rapidly with the six-bladed one than with the four-bladed. In the case of the six-bladed knife the number of capsules yielding latex at the sixth and seventh lancing proved a slight exception to this general statement but the number of heads then yielding latex was too small to draw conclusions from.

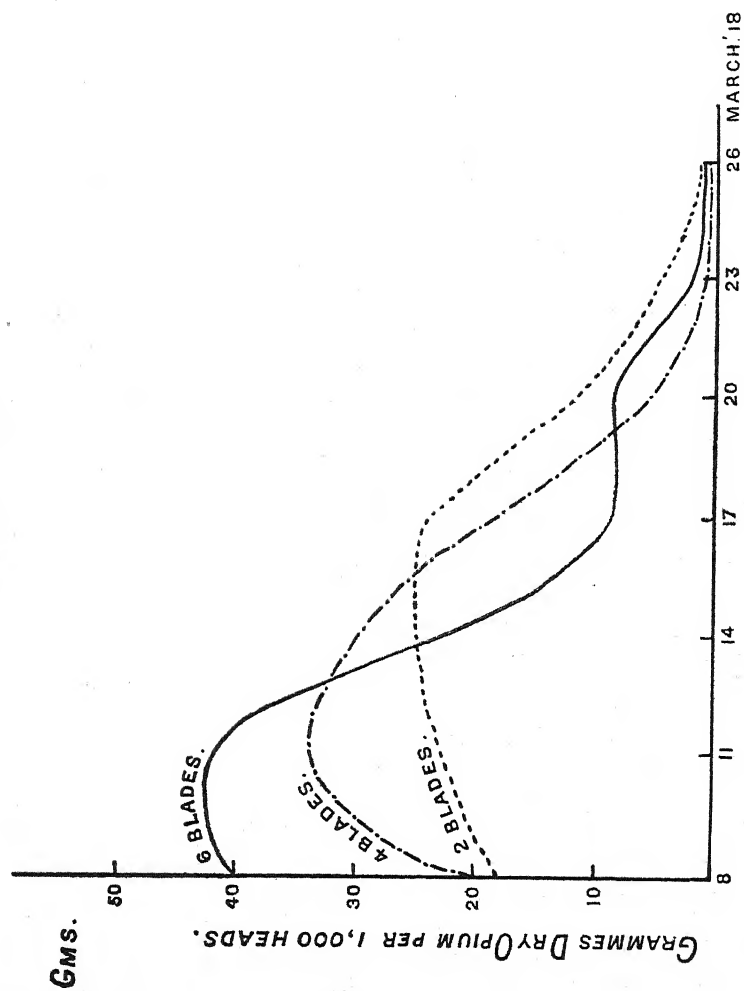
As regards the yield of opium (*see plate opposite*) the two-bladed knife has given a fairly steady yield at each of the first four lancings. With the four-bladed knife the second and third lancing showed a distinct increase in yield over the first and after that the yield quickly fell away. With six blades the yields per 1,000 capsules of the first two lancings were considerably higher than the corresponding yields with the two- and four-bladed knives and then the subsequent yields rapidly fell off. The total out-turns were 106.5, 108.4 and 124.0 gm. dry opium per 1,000 capsules from the use of 2- 4- and 6-bladed knives respectively.

As regards the morphine content of the opium of each successive lancing the third lancing with the six-bladed knife has produced opium of much lower morphine content than the corresponding lancing with two- and four-bladed knives.

A column has also been added to the table showing the total amount of morphine per 1,000 capsules removed in each case. Where a six-bladed knife was used it would appear that 50 per cent more morphine was obtained than in the case where a two-bladed knife was used.



YIELD OF OPIUM AS INFLUENCED BY  
THE NUMBER OF BLADES IN THE  
LANSING KNIFE.





*Effect of number of blades in the lancing knife. Cawnpore experiments 1918-19.*

Three groups, each of 2,000 terminal capsules in a fit condition for lancing, were selected. Each group was lanced with a 2- 4- or 6-bladed knife respectively. Successive lancements were carried out in each case until the capsules ceased to yield latex. The results are summarized below :—

Date of lancing	No. of lancing	No. of capsules lanced	No. of capsules yielding latex	Grm. dry opium	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium	Total morphine removed per 1,000 capsules grm.
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*Two-bladed knife.*

24-3-19	..	1st	2000	1993	88.62	44.31	11.8	5.23
27-3-19	..	2nd	2000	1781	62.13	31.06	7.8	2.42
30-3-19	..	3rd	2000	800	16.40	8.20	4.5	0.37
2-4-19	..	4th	320	154	4.10	2.05	0.0	0.00
5-4-19	..	5th	85	10	0.10	0.05	0.0	0.00
Total ..				171.35	85.67			8.02

*Four-bladed knife.*

24-3-19	..	1st	2000	1999	95.23	47.61	11.5	5.47
27-3-19	..	2nd	2000	1704	34.33	17.16	7.6	1.30
30-3-19	..	3rd	2000	528	11.60	5.80	4.7	0.27
2-4-19	..	4th	351	103	1.57	0.78	..	..
5-4-19	..	5th	53	10	0.16	0.08	..	..
Total ..				142.89	71.44			7.04

*Six-bladed knife.*

24-3-19	..	1st	2000	1998	85.38	42.69	11.8	5.04
27-3-19	..	2nd	2000	1658	39.51	19.75	8.4	1.66
30-3-19	..	3rd	2000	840	18.55	9.27	4.3	0.40
2-4-19	..	4th	310	195	5.99	3.00	2.5	0.07
5-4-19	..	5th	59	30	0.46	0.23	..	..
Total ..				149.89	74.94			7.17

These figures do not show any essential difference between the results obtained by using a knife containing 2, 4 or 6 blades. In this respect the results are not in agreement with those of the preceding and succeeding seasons.

*Cawnpore experiments 1919-20.*

These were carried out on the lines of the experiments of the two previous years and the results are set out in the table.

*Table showing the influence of using lancing knives containing 2, 4 and 6 blades respectively on the yield and composition of the opium from each successive lancing, 1919-20.*

Date of lancing	No. of lancing	No. of capsules lanced	No. of capsules yielding latex	Grm. dry opium	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium	Total morphine removed per 1,000 capsules grm.
Two-bladed knife.							
13-3-20	..	1st	(a) 527	521	20.82	39.51	6.45
			(b) 456	456	17.01	37.31	
			Average	..	38.41	16.8	
16-3-20	..	2nd	(a) 522	518	31.23	59.27	6.93
			(b) 436	436	19.82	43.46	
			Average	..	51.36	13.5	
19-3-20	..	3rd	(a) 500	470	10.34	19.61	2.13
			(b) 420	413	10.81	27.30	
			Average	..	23.45	9.1	
			Total ..	..	113.22	..	
Four-bladed knife.							
13-3-20	..	1st	(a) 493	493	15.29	30.87	6.33
			(b) 471	471	22.04	46.79	
			Average	..	38.83	16.3	
16-3-20	..	2nd	(a) 491	483	26.98	54.71	5.98
			(b) 471	469	26.37	56.01	
			Average	..	55.36	10.8	
19-3-20	..	3rd	(a) 479	424	12.15	24.66	1.65
			(b) 454	443	13.50	28.67	
			Average	..	26.66	6.2	
			Total ..	..	120.85	..	

Date of lancing	No. of lancings	No. of capsules lanced	No. of capsules yielding latex	Grm. dry opium	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium	Total morphine removed per 1,000 capsules gm.
<i>Six-bladed knife.</i>							
13-3-20	..	1st	(a) 540	540	48.21	89.30	15.1
			(b) 455	455	31.28	68.76	15.9
			Average	..	79.03	15.5	12.25
16-3-20	..	2nd	(a) 540	528	23.19	42.94	9.0
			(b) 455	444	16.84	37.00	10.1
			Average	..	39.97	9.5	3.80
19-3-20	..	3rd	(a) 538	461	14.50	26.85	5.8
			(b) 455	381	14.75	32.42	5.6
			Average	..	29.63	5.7	1.69
Total				..	148.63	..	17.74

As regards yield of opium there is a gradual increase in the total of all lancings with increasing number of blades in the knife. As between the two- and four-bladed knife there seems no marked difference in the yield at each successive lancing. The morphine content seems however to fall off more rapidly in the successive lancings in the case of the four-bladed knife. With six blades in the knife we get a largely increased yield at the first lancing and a regular decrease in each of the next two lancings. Moreover in the case of the six-bladed knife the percentage of morphine in the opium of the first lancing is distinctly less than where a two- or four-bladed knife was used. This probably is in sympathy with the largely increased yield of latex obtained at the first lancing with the six-bladed knife. In the opium of second and third lancings the morphine content is less in the case of the four-bladed knife than in that of the two-bladed knife. It is much less still in the case of the six-bladed knife. There does not seem in this year's experiment any marked increase of yield of total morphine corresponding to the increased number of blades in the knife as appeared to be indicated in the results for the season 1917-18.

#### 6. THE EFFECT OF THE TIME OF LANCING AND COLLECTION.

As already explained the usual method of harvesting opium is to lance the capsule shortly after midday, and to collect the opium next morning. We



decided to carry out experiments to see if lancing in the early morning and collecting the same evening had any influence on the quality or quantity of the resulting opium.

The first experiment was carried out at Douglas Dale in April, 1918. A pure race of poppy was employed.

On the morning of the 8th April, 1918, at about 8-30 a.m., 665 capsules (only single-capsuled plants were selected for the experiment) were lanced in the ordinary Indian fashion. The opium was collected that evening at about 5 p.m. Six subsequent lancements were made on these same capsules, all the lancements being made at 8-30 a.m. and the collection the same evening.

On the afternoon of the 8th at 2 p.m. (the usual lancing time) 579 capsules were lanced. The opium was collected next morning in the usual Indian fashion. Six subsequent lancements were made in exactly the same way. The dry matter and the morphine content of the opium of each lancing were then estimated. The accompanying table sets out all the results.

*The influence of time of day at which each lancing is carried out on the yield and morphine content of the opium produced.*

No. of lancing	Date of lancing	GROUP I LANCED MORNING, COLLECTED EVENING			GROUP II LANCED AFTERNOON, COLLECTED NEXT MORNING		
		No. of capsules lanced	Grm. dry opium per 1,000 capsules	Percentage of morphine in opium	No. of capsules lanced	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium
1	8-4-18	665	17.43	14.3	579	26.62	13.3
2	11-4-18	665	27.70	10.5	579	33.76	8.9
3	14-4-18	665	36.10	7.1	579	29.52	5.6
4	17-4-18	665	17.13	4.1	579	18.22	2.1
5	20-4-18	665	7.86	2.4	579	5.59	2.8
6	23-4-18	665	6.04	1.5	579	0.52	....
7	26-4-18	665	0.50	....	579	0.22	....
		Total ..	112.76	....	..	108.45	....

The experiments were repeated at Cawnpore in the season 1918-19. Three groups each of 1,000 terminal capsules at the correct stage for lancing were selected. The three groups were lanced at 9 a.m., 1 p.m. and 6 p.m.

respectively. The latex from the group lanced at 9 a.m. was collected at 6 p.m. the same day. The latex of the other two groups was collected at 6 a.m. next morning. The capsules received a second and third lancing at corresponding times at 3 days' intervals. At each lancing only one cut with a four-bladed knife was made. The table sets out the results.

Group	No. of lancing	Date of lancing	No. of capsules lanced	Grm. dry opium per 1,000 capsules	Percentage of morphine in dry opium
Group I, lanced 9 a.m., collected 6 p.m.	1	23-3-19	1000	27.54	12.4
	2	26-3-19	1000	30.72	9.8
	3	29-3-19	830	7.71	5.0
Total ..	..	..	..	65.97	..
Group II, lanced 1 p.m., collected 6 a.m. next day	1	23-3-19	1000	34.20	11.2
	2	26-3-19	1000	34.19	8.3
	3	29-3-19	800	11.37	2.0
Total ..	..	..	..	79.76	..
Group III, lanced 6 p.m., collected 6 a.m. next day	1	23-3-19	1000	37.68	11.6
	2	26-3-19	1000	24.83	8.0
	3	29-3-19	1000	5.61	3.2
Total ..	..	..	..	68.12	..

It appears therefore that the time of day at which the lancing is carried out has no important influence on the morphine content of the latex. Neither does it appear to have any marked influence on the yield of latex.

The yield of opium was therefore practically unaffected by the difference in time of lancing. The capsules lanced in the morning would appear to have produced opium at the first three lancements rather richer in morphine than that from the corresponding lancements of the capsules which were lanced in the afternoon. The difference however is not sufficiently great to indicate that the time of lancing has any effect on the morphine content of the resulting opium.

#### CONCLUSION.<sup>1</sup>

1. The Turkish method of spiral incisions gives no larger yield of opium than the Indian method of vertical incisions. It is moreover far more time-absorbing and more difficult to carry out than the Indian method. The same remarks apply to the case of transverse incisions.

2. As a rule there is no advantage to be gained by making more than one vertical incision at each lancing. The yield of latex is not proportionally

<sup>1</sup> See also pp. 21 and 27.

increased by increasing the number of incisions and if 5 or 6 vertical incisions are made the yield is actually less than with one incision. Usually three incisions per lancing gives a fair increase over a single incision at the first lancing, but the yield at the subsequent lancements falls off so much by the former method that the total yield of all the lancements is usually less by that method than by the latter.

As regards morphine content it would seem that if three incisions are made at each lancing the morphine content of the first lancing tends to be rather lower than that of the first lancing obtained by the ordinary method of single incisions. Moreover the falling off in morphine content of the opium from each subsequent lancing is much more rapid where three incisions are made at each lancing than where only one is made. It would seem that the capsule is only capable of yielding a fixed amount of morphine, and that if a large amount of latex is removed at the first lancing there is less morphine for the latex of the subsequent lancements.

3. When instead of making a full length incision at each lancing, the incision is only made one-third as long, or less, the yield of latex is considerably diminished at the first lancing. Our experiments however indicate that though the yield of latex is smaller at the first lancing yet the percentage of morphine in its dry matter is higher. Moreover the percentage of morphine in the opium from each succeeding lancing does not fall off so rapidly as it does when full length incisions are made at each lancing.

4. An experiment to test the effect of the number of blades in the knife used for lancing showed that a knife with six blades gave a bigger total yield of opium per capsule than a knife with four or two blades. At the first lancing the six-bladed knife may give double the yield of opium that was obtained by the use of the four- or two-bladed knife.

5. Our experiments indicate that there is no difference in the yield or morphine content of the opium produced by lancing in the early morning or in the afternoon.

Finally we would point out that much depends on the size of the capsule and the vigour of the plant. A large capsule of a vigorous plant can apparently receive more than one incision at each lancing without suffering much harm whilst a poorly nourished capsule may refuse to yield latex at all if more than one incision be made on it at each lancing.

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## INVESTIGATIONS ON INDIAN OPIUM, No. 2.

### THE EFFECT OF ENVIRONMENTAL FACTORS ON THE ALKALOIDAL CONTENT AND YIELD OF LATEX FROM THE OPIUM POPPY (*PAPAYER SOMNIFERUM*) AND THE BEARING OF THE WORK ON THE FUNCTIONS OF ALKALOIDS IN PLANT LIFE.

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#### INTRODUCTION.

THE writer in collaboration with others has described the manner in which alkaloid content and yield of poppy latex are influenced by factors of a non-environmental nature. The effects there considered were practically all connected with the methods of lancing and collection adopted. In this memoir it is proposed to give an account of the effect of certain environmental factors on the alkaloidal content and yield of latex. In this connection the effects of manures, climate, season and of weather conditions are considered. The stage of development of the capsule yielding latex is practically an environmental factor, and is included in this account. The number of capsules on a plant depends to a large extent on environment, and, as will be shown below, the capsule first produced by the plant produces latex of a very different composition from that of the latex from the capsules subsequently appearing. When, therefore, one is dealing with effects due to weather conditions, manures or age of capsules, it is important to confine one's attention to the latex produced by the oldest capsule only of each plant. Because of this, it is

proposed to deal first with the yield and composition of the latex of the oldest and of the younger capsules of each plant. In order to follow the account of the work, the description of the method of lancing the capsules for opium should be read.<sup>1</sup>

#### THE INFLUENCE OF THE NUMBER OF CAPSULES ON THE PLANT.

The writer has observed as many as 40 capsules in various stages of development on a plant of the opium poppy. Usually, however, the plants have one or two, or, perhaps, three capsules. The oldest is at the end of the main stem. The subsequent capsules are borne each at the end of a branch arising from an axil of a leaf on the main stem, and the younger the capsule the further down the main stem does its branch originate. Moreover, the youngest lateral capsule overtops all the rest, so that the original, or terminal, capsule can generally easily be recognized from the fact that it is overtopped by all the others. It seemed of interest to determine if there were any differences to be observed between the yield and composition of the opium obtained from the oldest capsules on a plant, and from those subsequently appearing. The following experiment was carried out at Almorah in the Himalayas. A number of plants, with at least 5 capsules each, were taken in such a stage that their terminal capsules were ready for lancing. These latter were lanced. In the opium produced estimations were made of the total dry matter and of the percentage of morphine.

Three days later, the next oldest capsules on these plants, *i.e.*, the first lateral capsules, were lanced. Only 146 were ready for lancing. These will be referred to as the first lateral capsules. Three days later the next oldest capsules on the plants were lanced : 334 were taken. These are designated the second lateral capsules. Three days later the third lateral capsules, 120 in number, were lanced. The varying numbers of the terminal, 1st, 2nd and 3rd lateral capsules lanced were due of course to the fact that, though the terminal capsules in all the plants were ready on the same day, because they were chosen for this reason, the 1st lateral capsules of all the plants were not ready on one day and so on. In each case, as many subsequent lancements were applied as the capsules would bear. The results are set out in the following table :—

<sup>1</sup> Annett, Sen and Singh. *Mem. Dept. Agri. India (Chem. Series)*, vol. VI, no. 1 (pt. II).

No. of lancing	TERMINAL CAPSULES					LATERAL CAPSULES				
	Date of lancing	No. of capsules lanced	Weight of dried opium (gram.)	Weight of dried opium per 1,000 capsules (gram.)	Per cent. of morphine in dry matter of opium	Date of lancing	No. of capsules lanced	Weight of dried opium (gram.)	Weight of dried opium per 1,000 capsules (gram.)	Per cent. of morphine in dry matter of opium
1st	20-4-17	491	16.25	33.10	11.9	23-4	146	2.70	18.50	7.30
2nd	23-4-17	491	18.80	38.29	9.57	26-4	146	1.95	13.36	6.04
3rd	26-4-17	491	9.25	18.44	5.81	29-4	146	1.35	9.25	—
4th	29-4-17	491	5.70	11.61	3.64	2-5	146	1.65	11.30	—
5th	2-5-17	491	12.45	25.35	2.16	10-5	71	1.00	14.06	—
6th	10-5-17	251	3.15	12.55	1.59	12-5	40	0.30	7.5	—
7th	12-5-17	119	0.80	6.72	—	—	—	—	—	—

The results are very definite. Dealing, firstly, with the yield of dry opium per 1,000 capsules, it will be seen that the terminal capsules are much the heaviest yielders. They gave nearly twice as much opium at the first lancing as the lateral capsules, and 3 to 4 times as much at the second lancing.

Turning to the morphine content in the dry opium, one finds that at each lancing it is far greater in the case of the terminal than in the lateral capsules. In the season 1917-18 further investigations on these lines were carried out. A number of plants with three capsules, of which the terminal was ready for lancing, were selected on the same day, *viz.*, 2nd March, 1918. Each was marked with a piece of red cloth, so that they could easily be found.

The terminal capsules were lanced on that day, and counted, and the opium collected next morning. These capsules received further lancements on the 5th, 9th, and 12th March. On the 5th March, any lateral capsules ready for lancing, namely, 1,053, were lanced and the opium collected next day. These subsequently received a second, third, and fourth lancing on the 9th, 12th and 15th March. On the 9th March, a fresh lot of lateral capsules were ready for lancing. These received a second and a third lancing on the 12th and 15th March. On the 12th March, still more lateral capsules were ready for lancing. These received a second lancing on the 15th March. The number of heads of each lancing was counted and the yield of dry opium and its morphine content were estimated. The results are summarized in the table.

Sample	No. of lancing	Date of lancing	No. of capsules yielding latex	Grm. opium (dried in steam oven)	Grm. opium per 1,000 capsules	Percentage of morphine in dry matter of opium
Terminal capsules	1st	2-3-18	1,620	129.80	79.7	14.0
	2nd	5-3-18		43.76	27.0	8.4
	3rd	9-3-18		13.34	8.2	5.3
	4th	12-3-18		5.70	3.5	3.3
Lateral capsules	1st	5-3-18	1,053	36.39	34.6	12.2
	2nd	9-3-18		8.40	8.0	7.1
	3rd	12-3-18		2.24	2.2	4.9
	4th	15-3-18		0.30	0.3	..
Lateral capsules, 2nd crop	1st	9-3-18	1,563	21.52	13.7	10.1
	2nd	12-3-18		10.10	6.5	6.1
	3rd	15-3-18		1.10	0.7	..
Lateral capsules, 3rd crop	1st	12-3-18	193	2.77	14.3	3.0

In the season 1917-18, a further experiment on the same lines was carried out in the Kumaun Hills at Douglas Dale near Naini Tal. A number of plants having more than one capsule were taken. In order to have duplicate results they were divided into two roughly equal groups, the actual number of plants in each group being 407 and 376.

The terminal capsule and also the oldest lateral capsule on each plant were lanced. The table sets out all the results.

	NUMBER OF LANCING									
	First		Second		Third		Fourth		Fifth	
	Grm. dry opium per 1,000 capsules	Per cent. morphine in dry opium	Grm. dry opium per 1,000 capsules	Per cent. morphine in dry opium	Grm. dry opium per 1,000 capsules	Per cent. morphine in dry opium	Grm. dry opium per 1,000 capsules	Per cent. morphine in dry opium	Grm. dry opium per 1,000 capsules	Per cent. morphine in dry opium
407 terminal capsules of 407 plants	31.4	15.7	39.8	12.8	33.1	7.5	17.9	9.4	19.5	2.8
407 lateral capsules of same plants	12.8	8.2	18.1	5.0	15.3	1.6	10.5	1.3	9.1	0.0
376 terminal capsules of 376 plants	20.1	16.7	27.4	13.4	35.6	8.1	38.6	5.9	17.5	0.0
376 lateral capsules of same plants	9.2	7.7	19.7	6.8	19.0	3.2	20.0	2.8	8.5	0.0

The table brings out very clearly the low morphine content of the opium from lateral capsules. Moreover, the morphine content of opium from the subsequent lancements falls off much more rapidly than it does in the cases of terminal capsules. Further, the yield of opium from the lateral capsules is far smaller than from the terminal capsules.

It appeared to be of interest to determine whether lateral capsules would give opium of higher morphine content if the terminal capsules on the same plants were left unlanced. Accordingly, about 500 plants with two or more capsules, but whose terminal capsules were all ripe for lancing, were selected and labelled with red cloth labels. About 500 plants on the same plot of the same pure race having two or more capsules, but on which a lateral capsule was ready for lancing, were also selected on the same day and distinguished with paper labels.



The terminal capsules of the red labelled plants and one mature lateral capsule on each of the paper-labelled plants were then lanced on that same day. Three simultaneous cuts per head were made at each lancing instead of the usual one. Unfortunately, the result of this was that only two lancements were obtained and the yield of the second lancing was too small for analysis in the case of the lateral capsules.

The results are set out below :—

Groups	No. of lancing	Date of lancing	Grm. opium (dried in steam oven)	No. of capsules yielding latex	Dry opium per 1,000 capsules gm.	Percentage of morphine in dry opium
RED LABELS						
Terminal capsules lanced	1st	8-3-18	44.52	456	97.2	15.0
	2nd	11-3-18	7.08	224	15.5	7.1
PAPER LABELS						
Lateral capsules only lanced	1st	8-3-18	30.77	462	55.8	12.4
	2nd	11-3-18	1.63	64	3.6	..

These results indicate that even when the terminal capsules are unlanced, the lateral capsules yield much less opium, and also the opium contains less morphine. The result is extremely interesting, inasmuch as it would indicate that the latex most concentrated in morphine cannot be attracted away from the terminal capsules.

Arrangements were therefore made to investigate this point more thoroughly in the season 1918-19. The writer is indebted to Dr. J. N. Sen for carrying on these experiments to a successful conclusion during the former's sudden illness. A field measuring 0.56 acre was selected, on which a pure race of poppy was growing. It was divided into 5 plots, A, B, C, D, and E. For the purpose of the experiment, plants were selected which had more than one capsule each. The following procedure was then adopted in each of the plots.

*Plot A.* Only the terminal capsule on each plant was lanced. The lateral capsules were left on the plants unlanced.

*Plot B.* Only lateral capsules were lanced and the terminal capsules were left on the plants unlanced.

*Plot C.* The terminal capsules were lanced, all lateral capsules having been removed from the plants just previously.

*Plot D.* Lateral capsules only were lanced, the terminal capsules on the same plants having been removed just previous to lancing.

*Plot E.* Both terminal and lateral capsules on the same plants were lanced but the produce of the former was kept separately from that of the latter. Naturally, in this case, the lateral capsules were not ready for lancing till some days after the lancing of the terminal capsules.

In each of the above cases five successive lancings of the capsules were made, and at each lancing only one incision with a four-bladed knife was made. The results are tabulated below.

Description of experiment	No. of lancing	No. of heads lanced	Yield of opium (dry matter) gm.	Yield per 1,000 capsules gm.	Per-centage of mor-phine in dry opium
A					
Terminal capsules lanced, unlanced laterals left on plant	1st 2nd 3rd 4th 5th	807	18.15 41.35 45.52 14.51 2.90	22.48 51.25 57.65 18.00 3.59	14.5 11.6 7.8 4.0 2.7
TOTAL			122.43	152.97	
B					
Lateral capsules lanced, unlanced terminals left on plant	1st 2nd 3rd 4th 5th	832	40.24 43.20 22.42 2.14 0.40	48.36 51.92 25.74 2.57 0.48	11.8 9.2 6.1 4.4 ..
TOTAL			108.40	129.07	
C					
Terminal capsules, lanced, unlanced laterals previously removed	1st 2nd 3rd 4th 5th	1,942	100.68 96.72 58.87 26.25 0.80	51.85 49.80 30.31 13.51 0.41	14.4 10.7 7.3 5.4 ..
TOTAL			283.32	145.88	
D					
Lateral capsules lanced, unlanced terminal previously removed	1st 2nd 3rd 4th 5th	1,970	110.16 112.73 34.09 12.70 0.45	55.91 57.21 17.26 6.40 0.23	10.9 7.2 5.0 2.6 ..
TOTAL			270.13	137.01	

Description of experiment	No. of lancing	No. of heads lanced	Yield of opium (dry matter) grm.	Yield per 1,000 capsules grm.	Percentage of morphine in dry opium
E					
Both terminal and lateral capsules on same plants lanced	Terminal				
	1st		40.28	65.71	13.6
	2nd		39.15	63.86	9.0
	3rd	613	13.64	22.25	4.4
	4th		0.91	1.60	..
	5th		0.19	0.40	..
			94.17	153.82	..
TOTAL					
	Lateral				
	1st		22.82	31.14	8.8
	2nd		25.50	34.80	5.2
	3rd	733	2.43	3.35	..
	4th		0.49	0.68	..
	5th		0.31	0.42	..
			51.55	70.31	
TOTAL					

AS regard: morphine content of the opium produced, the foregoing table shows that the lateral capsules produce poorer opium than the terminal capsules do, whether the terminal capsules on the same plants are removed or not. When, as in plot E, the terminal capsules have been lanced, the opium of the lateral capsules, lanced later, in the same plants is of particularly low morphine content. Turning to the yield of opium it appears that the removal of lateral capsules, previous to lancing the terminal capsules, does not result in any increased yield of latex from the terminal capsules. Neither is the yield from the terminal capsules increased by leaving the lateral capsules unlanced. Similarly the yield of latex from the lateral capsules is not increased by leaving unlanced the terminal capsules or removing them altogether. When, however (*see* plot E), the terminal capsules are first lanced, the yield of latex subsequently obtained by lancing the lateral capsules on the same plants appears to be considerably diminished.

Experiments were carried out in the season 1917-18 to check the foregoing results on a large scale at Cawnpore. Mr. Leake had many hundreds of pure races of poppy growing in small plots. These were being grown so that we could examine the morphine content of opium of each with a view to the isolation of races of poppy giving opium of high morphine content. On certain of these plots all the plants had large numbers of capsules. On 50 such plots the terminal capsules were first lanced and the number of capsules

lanced counted. When sufficient lateral capsules were ready for lancing they were also lanced and counted. In all the plots more terminal capsules came to maturity for lancing after the first lancing, and these were again counted and lanced. The opium of each collection was then examined for loss on drying in the water oven, and for morphine content. The table expresses the results in grm. of dry opium per 1,000 capsules, and also states the percentage of morphine in the opium produced.

Plot No.	TERMINAL CAPSULES					LATERAL CAPSULES				
	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-centage of mor-phine in dry opium	Grm. dry opium per 1,000 cap-sules	Date of lancing	Grm. dry opium	No. of cap-sules lanced	Per-centage of mor-phine in dry opium	Grm. dry opium per 1,000 cap-sules
148	26-2-18	16.60	201	12.5	83.9	5-3-18	21.60	389	9.6	55.5
	4-3-18	21.73	96	12.5	220.6					
149	26-2-18	15.75	228	12.3	69.1	5-3-18	28.25	410	10.8	69.0
	4-3-18	19.07	286	12.3	66.7					
150	26-2-18	2.83	67	11.9	42.5	5-3-18	13.98	191	10.5	73.2
	4-3-18	26.20	466	12.3	56.2					
151	28-2-18	13.44	164	12.4	82.0	5-3-18	14.37	231	10.5	62.2
	4-3-18	13.54	240	12.4	56.3					
158	26-2-18	18.61	224	12.0	83.8	5-3-18	13.76	169	9.2	81.4
	4-3-18	18.76	338	12.7	55.8					
159	28-2-18	21.72	241	10.6	90.1	5-3-18	12.12	123	9.4	98.5
	4-3-18	26.35	511	18.2	49.5					
160	28-2-18	36.36	411	10.8	88.5	5-3-18	23.96	423	8.7	56.7
	4-3-18	23.90	421	12.4	56.8					
161	28-2-18	7.97	140	10.2	57.0	5-3-18	11.46	224	10.0	51.1
	4-3-18	1.80	11	..	164.0					
162	28-2-18	45.25	598	12.8	75.7	5-3-18	24.75	564	9.9	43.9
	4-3-18	10.30	160	11.8	64.4					
163	28-2-18	32.18	374	12.6	86.0	5-3-18	25.20	551	10.1	45.7
	4-3-18	15.5	329	12.3	47.3					

Plot No.	TERMINAL CAPSULES					LATERAL CAPSULES				
	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-cent- age of mor- phine in dry opium	Grm. dry opium per 1,000 capsules	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-cent- age of mor- phine in dry opium	Grm. dry opium per 1,000 capsules
164	28-2-18	34.90	306	12.3	114.1	5-3-18	21.86	351	10.3	62.3
	4-3-18	14.59	267	11.5	54.7					
165	28-2-18	20.72	296	13.4	70.0	5-3-18	18.03	306	11.5	58.9
	4-3-18	12.75	286	13.3	44.7					
166	28-2-18	24.53	475	13.0	51.7	5-3-18	21.67	229	10.5	94.6
	4-3-18	8.01	312	12.8	25.6					
167	28-2-18	19.22	202	11.7	95.1	5-3-18	15.13	266	10.5	56.9
	4-3-18	19.36	421	11.9	46.1					
168	28-2-18	18.92	257	11.8	73.6	5-3-18	11.83	210	8.8	56.3
	4-3-18	19.32	341	11.5	56.6					
169	28-2-18	23.24	394	12.6	59.0	5-3-18	12.75	239	10.5	53.3
	4-3-18	10.33	333	12.1	31.0					
170	28-2-18	31.15	358	12.1	87.0	5-3-18	25.70	505	9.5	50.9
	4-3-18	14.28	268	11.8	53.3					
171	28-2-18	31.78	382	12.0	83.0	5-3-18	22.98	439	9.9	52.3
	4-3-18	11.92	271	12.0	44.0					
172	28-2-18	22.00	181	12.8	121.5	5-3-18	16.14	146	10.5	110.6
	4-3-18	21.17	400	13.0	50.3					
173	28-2-18	34.89	430	12.0	81.2	5-3-18	22.70	358	10.3	63.4
	4-3-18	4.68	297	11.6	15.6					
174	28-2-18	5.93	60	11.4	98.9	5-3-18	26.07	214	10.5	121.8
	4-3-18	8.20	275	12.0	30.0					
175	19-2-18	9.86	291	12.6	33.9	5-3-18	22.63	568	9.5	39.8
	4-3-18	17.64	102	12.5	175.0					
176	19-2-18	6.26	200	13.5	31.3	5-3-18	19.00	579	12.2	32.8
	4-3-18	5.60	182	13.1	31.0					



Plot No.	TERMINAL CAPSULES					LATERAL CAPSULES				
	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-cent- age of mor- phine in dry opium	Grm. dry opium per 1,000 capsules	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-cent- age of mor- phine in dry opium	Grm. dry opium 1,000 per capsules
177	19-2-18	3.22	73	11.9	44.8	5-3-18	27.83	594	10.2	46.8
	4-3-18	31.16	391	12.8	80.0					
178	26-2-18	34.55	596	10.7	58.0	5-3-18	21.63	646	8.5	33.5
	4-3-18	10.31	..	13.1	..					
179	26-2-18	34.38	591	11.4	58.2	5-3-18	14.20	459	9.4	30.7
	4-3-18	8.02	262	12.5	30.6					
180	26-2-18	21.91	713	12.9	30.7	5-3-18	14.76	376	10.9	39.2
	4-3-18	4.15	163	12.0	25.4					
181	26-2-18	21.86	674	13.1	32.4	5-3-18	9.84	317	9.9	31.0
	4-3-18	5.53	261	13.0	21.2					
182	26-2-18	26.02	598	12.5	44.0	5-3-18	11.49	291	10.1	39.5
	4-3-18	6.34	259	12.7	24.1					
183	28-2-18	20.42	580	12.2	35.2	5-3-18	9.60	343	9.7	28.0
	4-3-18	6.08	229	12.5	27.0					
184	28-2-18	17.56	319	11.7	55.1	5-3-18	12.40	343	9.5	36.2
	4-3-18	18.65	310	12.4	60.1					
185	28-2-18	14.80	360	11.1	41.2	5-3-18	10.28	686	9.3	15.0
	4-3-18	3.60	89	9.2	40.4					
186	28-2-18	41.32	599	12.0	69.0	5-3-18	19.14	294	9.4	65.2
	4-3-18	7.80	194	11.8	40.2					
187	19-2-18	8.57	422	14.7	20.3	5-3-18	14.96	487	12.3	30.7
	4-3-18	9.31	295	13.2	31.6					
188	19-2-18	8.23	221	13.0	36.7	5-3-18	15.7	503	10.9	31.2
	4-3-18	11.6	362	13.4	31.8					
189	19-2-18	6.81	123	12.4	56.2	5-3-18	21.5	534	10.2	40.3
	4-3-18	12.7	384	12.2	33.1					

Plot No.	TERMINAL CAPSULES					LATERAL CAPSULES				
	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-centage of mor-phine in dry opium	Grm. dry opium per 1,000 capsules	Date of lancing	Grm. dry opium	No. of capsules lanced	Per-centage of mor-phine in dry opium	Grm. dry opium per 1,000 capsules
190	27-2-18	15.42	257	10.6	60.0	5-3-18	14.42	354	8.8	40.8
	4-3-18	4.07	118	10.8	34.5					
191	27-2-18	23.79	369	10.9	64.4	5-3-18	13.08	304	9.4	43.0
	4-3-18	6.65	204	10.8	32.1					
192	26-2-18	15.85	291	10.9	54.4	5-3-18	7.15	292	8.1	33.4
	4-3-18	11.85	277	10.3	42.8					
193	26-2-18	12.55	312	10.8	40.2	5-3-18	14.18	453	9.7	31.3
	4-3-18	6.02	350	10.9	17.2					
194	26-2-18	8.76	122	11.4	71.8	5-3-18	15.08	435	8.9	34.6
	4-3-18	16.70	459	11.3	36.4					
195	27-2-18	17.81	500	11.8	35.6	5-3-18	17.64	437	9.6	40.3
	4-3-18	2.10	131	11.1	16.0					
196	27-2-18	13.97	540	12.8	25.9	5-3-18	9.50	278	9.7	34.1
	4-3-18	2.40	114	10.7	21.1					
197	27-2-18	26.47	526	12.1	50.3	5-3-18	9.44	303	9.2	31.1
	4-3-18	4.97	203	11.9	24.5					
198	27-2-18	18.32	556	12.4	33.0	5-3-18	11.19	475	11.7	23.6
	4-3-18	0.80	83	..	9.6					
199	27-2-18	32.06	493	11.8	65.2	5-3-18	17.60	552	9.0	31.9
	4-3-18	9.34	314	11.6	29.4					
200	27-2-18	21.69	480	11.5	45.0	5-3-18	11.94	440	10.4	27.2
	4-3-18	8.60	132	10.8	..					
201	27-2-18	13.15	194	9.5	67.8	5-3-18	10.03	492	9.0	20.4
	4-3-18	1.80	79	..	22.8					
202	27-2-18	17.87	225	11.7	79.4	5-3-18	15.17	413	9.5	36.7
	4-3-18	4.20	105	11.1	40.0					
203	27-2-18	16.31	181	10.2	90.0	5-3-18	20.72	459	10.0	45.1
	4-3-18	5.94	132	10.8	45.0					

The above table affords ample confirmation of the fact that lateral capsules on a plant produce opium of lower morphine content than the terminal capsules do. Out of the 50 plots examined not one has given opium from its lateral capsules higher in morphine content than that from its terminal capsules. The lancing of terminal capsules which appeared on later maturing plants in each plot provided a check on the experiment. The opium from both crops of terminal capsules showed, in the majority of cases, practically identical morphine content and this does away with the possibility that the lateral capsules gave opium of lower morphine content because they were lanced later in the season. Moreover, it will be seen later that, after the capsule has passed the soft stage, practically no change takes place in the morphine content of the latex from the first lancing.

As regards the yield of opium from the lateral capsules the figures are not so conclusive. Certainly, in the majority of cases, the lateral capsules seem to have yielded less opium than the terminal ones, but there are, on the other hand, many cases in which the lateral capsules have given a greater yield than the terminal ones.

It would seem that by removing all lateral capsules from a plant the yield of latex from the terminal capsule is increased. This is borne out by the following experiment carried out in 1917-18. An area of  $\frac{1}{2}$  acre was divided at flowering time into 4 equal plots (a), (b), (c), and (d). On plots (a) and (c) all lateral flower buds were removed as soon as they were formed, whereas on plots (b) and (d) the plants were allowed to produce as many capsules as they were able to. On the occasion of the first lancing, all the capsules lanced in each plot were counted. The capsules received three vertical cuts at each lancing and three successive lancements were carried out. The table sets out the results.

No. of lancing	PLOT (a)		PLOT (b)		PLOT (c)		PLOT (d)	
	ALL LATERAL BUDS REMOVED		NO BUDS REMOVED		ALL LATERAL BUDS REMOVED		NO BUDS REMOVED	
	Total dry opium per 1,000 capsules grm.	Per-cent-age of mor-phine in dry opium	Total dry opium per 1,000 capsules grm.	Per-cent-age of mor-phine in dry opium	Total dry opium per 1,000 capsules grm.	Per-cent-age of mor-phine in dry opium	Total dry opium per 1,000 capsules grm.	Per-cent-age of mor-phine in dry opium
1st	83.3	14.1	51.0	14.2	70.3	14.6	46.3	13.9
2nd	22.6	7.8	18.4	9.7	31.6	9.7	10.7	10.7
3rd	6.9	6.6	6.5	5.4	19.6	4.8	6.0	6.6

The yield of opium per 1,000 capsules is in each case much greater from the plots in which all lateral buds were picked off, this being especially marked in the product of the first lancing. It may, of course, be that a number of lateral capsules were included in the first lancements of plots (b) and (d). Since it has been shown that lateral capsules usually yield less opium than terminal ones, this might account for the lower yield per 1,000 capsules in plots (b) and (d). Unfortunately no record was made of the number of lateral capsules included in the lancements of plots (b) and (d). Since the lateral capsules come to maturity later than the terminal ones in the same plant, it is improbable that there could have been enough lateral capsules lanced in plots (b) and (d) to account for such a large difference in yield from that in plots (a) and (c). It therefore seems strongly indicated that the removal of lateral buds results in a bigger yield of latex by the remaining terminal capsules. If one aims at single-capsuled plants, it is therefore likely that they will be good yielders. This is an important point from the point of view of the production of high morphine content opium.

#### CONCLUSIONS.

1. The terminal or oldest capsules of a plant produce opium of considerably higher morphine content than the lateral capsules do. Hence to produce opium of high morphine content, one should endeavour to grow plants with single capsules. For any particular plant bearing many capsules the indications are that the morphine content of its opium falls off in the order of origin of the capsules.

It would appear that the inferiority of the opium from lateral capsules in morphine content has not been discovered by the Turks. In the *Encyclopædia Britannica*<sup>1</sup>, it is stated with reference to Turkey that "the capsules are generally incised only once but the fields are visited a second or third time to collect the opium from the poppy heads subsequently developed by the branching of the stem."

2. As regards the out-turn of opium, terminal capsules usually produce more opium than lateral capsules and this is a further reason for growing plants with only a single capsule. By removing lateral capsules it would appear that the opium yield of the remaining terminal capsules can be considerably increased. Since it is shown later, in the account of the effect of manure on the alkaloidal content of opium, that, for a particular race of poppy, the yield of opium is proportional to the size of the capsule, the present conclusion

<sup>1</sup> Article on "Opium," 11th Edn., page 131.

is what one might expect. By removing lateral capsules the remaining terminal one might be expected to reach a larger size and in this way the larger yield of opium would be accounted for.

### THE INFLUENCE OF THE AGE OF THE CAPSULES.

It has been shown previously<sup>1</sup> that, when a capsule is lanced on consecutive days, or on every second or third day, the opium of the first lancing is richest in morphine, and that the opium from each successive lancing shows a regular decrease in its morphine content. It was there remarked (p. 15) that a possible explanation of this falling off in morphine content of the opium of each successive lancing might be that the morphine content of the capsule decreased during ripening. The work here described will, however, show that this explanation is inadmissible.

A review of the literature indicates that a certain amount of work has been done on the alkaloidal content of poppy capsules, at various stages of ripeness. Malin<sup>2</sup> carried out some experiments to determine the alkaloidal content of ripe and unripe poppy capsules. The stage of ripeness is not clearly defined. Malin states that the unripe capsules taken were from two harvests of 12th and 16th August 1905 respectively. The former were still distinctly green and those of the second harvest were scarcely noticeably riper. Malin also took unlanced ripe capsules from the fields on 17th September. Using somewhat unsatisfactory methods of analysis he obtained the following results :—

Date of harvesting	Percent- age of morphine	Percentage of narcotine plus codeine
Unripe capsules harvested 12th August . . . . .	0.050	0.0113
„ „ „ 16th August . . . . .	0.020	0.0116
Fully ripe „ „ 17th September . . . . .	0.018	0.0280

Malin considers that these figures show that, during the ripening of the poppy capsule, the morphine diminishes, and the narcotine and codeine increase.

<sup>1</sup> Annett, Sen, and Singh. *Mem. Dept. Agri. India, Chem. Ser.*, vol. VI, no. 1 (pt. III).

<sup>2</sup> Thoms. *Ueber Mohnbau und Opiumgewinnung*, p. 59, Berlin, 1907.



Tromsdorff<sup>1</sup> states that one year old ripe capsules contain neither morphine nor narcotine. Winkler<sup>2</sup> states that morphine and narcotine are present in fully ripe poppy capsules.

Merck<sup>3</sup> obtained 18 grains of morphine from 32 oz. of poppy capsules (roughly 0.0075 per cent.). Caesar and Loretz<sup>4</sup> in 1901 found 0.0189 per cent. morphine in fully ripe poppy capsules.

Muller<sup>5</sup> in an interesting paper states that, in plants grown under natural conditions, the amount of alkaloid in the capsules, stems, and leaves, diminishes, but does not completely disappear as the seed ripens. When the plants were removed from the soil at the flowering stage, and then grown in non-nitrogenous nutrient solutions, the alkaloidal content of the whole plant had diminished after 10 days. After 48 days, when the seed had ripened, the leaves and stems were quite free from alkaloids, and the capsule walls contained traces of alkaloids which could only be detected qualitatively, and were too small to be estimated.

The present writer has examined capsules which were harvested at the lancing stage but without having been lanced. They were dried in the sun, and the air-dried capsules after one year's storage yielded 0.645 per cent. of morphine and 0.018 per cent. of codeine calculated on the dry matter. The amount of alkaloids found depends much on the methods of investigation used. Accurate methods of estimating small amounts of morphine, codeine or narcotine in large quantities of plant material have been worked out in the writer's laboratory, and a description of them is being published elsewhere.

It is of interest that, where poppies are grown for their capsules, *e.g.*, in the Isle of Axholme in England, they are harvested well before they are dead ripe.

It is perhaps worth while to refer here to some of the literature bearing on the alkaloidal content of certain other medicinal plants at various stages of ripeness. In the *Year Book of Pharmacy* for 1914<sup>6</sup> the following passage occurs:—

“Farr and Wright's results seem to indicate that coniine present in hemlock may take part in the formation of the proteid reserve food found in the fruit. The amount of alkaloid found in the root, stem and leaves is small,

<sup>1</sup> *Archiv der Pharm.*, XXXVIII, p. 231.

<sup>2</sup> *Ann.* IV, page 237. Through Thoms. *loc. cit.*, p. 60.

<sup>3</sup> *Ann.* IV, page 237. Through Thoms. *loc. cit.*

<sup>4</sup> Thoms. *loc. cit.*, p. 60.

<sup>5</sup> *Archiv der Pharm.* 252, 1914, pp. 280-292.

<sup>6</sup> Presidential address, p. 314.

while in the fruit it is considerable during the period when the fruit is forming its reserve material, reaching as much as 3 per cent. or even more. When the fruit has finished forming its reserve and ripens, the proportion of alkaloid is found to become less, not greater, until it falls off to less than 1 per cent. in the ripe fruit. Moreover, the proportion of alkaloidal to total nitrogen gradually diminishes as the fruit develops. If the alkaloid were a by-product in the production of proteid, it might be expected to retain a fairly constant ratio and not become a diminishing one."

Carr<sup>1</sup> states that, when the belladonna plant begins to fade, there is a rapid loss of alkaloid in the stem and leaves.

Carr<sup>2</sup> also records cases showing the influence of advance of season on the amount of active principles in certain drugs. The following results were obtained with broom tops gathered month by month from the same locality throughout the year :—

Month	Percentage of sparteine sulphate
August	0·07
September	0·17
October	0·34
November	0·48
December	0·56
January	0·36
February	0·36
March	0·53
April	0·44
July	0·23

Carr concludes that there is a rapid loss of alkaloid during the flowering and growing period, which is slowly replaced during the quiescent months of autumn and winter.

#### EXPERIMENTAL.

At Almorah in the season 1916-17 two sets of plants were marked, one with capsules a little beyond the stage at which the cultivators take them

<sup>1</sup> International Congress of Applied Chemistry. "Effect of Cultivation on Alkaloidal Content of Belladonna." Through *Chem. and Drugg.*, 1912, 81, p. 432.

<sup>2</sup> Carr and Reynolds. *Pharm. Journ.*, IV, 26, p. 543.

for lancing, the other with the capsules green and soft, that is, at least a week earlier than the cultivators would use them. There were 499 plants in the first group, and 498 in the second. The capsules were lanced in the ordinary way and each received subsequent lancements as long as they yielded opium. The date of lancements and other details are set out in the table.

No. of lancing	IMMATURE CAPSULES					OVERMATURE CAPSULES				
	Date of lancing	No. of capsules lanced	Dried opium grm.	Dried opium per 1,000 capsules grm.	Per-cent- age of mor- phine in dry opium	Date of lancing	No. of capsules lanced	Dried opium grm.	Dried opium per 1,000 capsules grm.	Per-cent- age of mor- phine in dry opium
1st	20-4-17	498	6.80	13.60	8.94	20-4-17	499	12.40	24.85	13.28
2nd	23-4-17	498	5.55	11.14	6.44	2-5-17	499	10.55	21.14	10.20
3rd	26-4-17	498	5.60	11.24	4.61	10-5-17	342	4.40	12.87	5.01
4th	27-4-17	498	2.80	5.82	1.70	12-5-17	226	2.10	9.29	2.85
5th	2-5-17	498	3.50	7.03	1.25					
6th	10-5-17	290	2.02	6.96	1.25					
7th	12-5-17	190	1.05	5.52						

The results obtained would indicate fairly definitely that, if the capsules are taken in too young a stage, not only do they yield appreciably less opium, but this opium contains much less morphine reckoned on the dry substance. One must be very cautious in drawing general conclusions from a single experiment in this kind of work, for there may be so many other factors involved, *e.g.*, weather conditions for one, and it was not possible to find sufficient plants to make up two groups on the same day.

#### 1917-18 EXPERIMENTS.

In the season 1917-18 arrangements were made to investigate the questions more in detail at Cawnpore. The plan of this experiment was to collect opium from capsules in different stages of development. In order to do this, it was necessary to make sure of the age of the capsules chosen. The field used had been sown with a pure race of poppy. Its area was 0.85 acre. The original idea was to take some 20,000 plants in this field which were at the same stage on a particular day. The stem bearing the bud straightens out in the case of the poppy the day before the flower opens, and the buds were selected at this stage. As far as possible plants showing only one bud were chosen.

Any lateral buds subsequently appearing were carefully removed. These 20,000 plants were to be divided into six groups of roughly 3,000 plants each, and the plants of each group labelled with cloth labels of a distinguishing colour for each group. It was then proposed to collect opium from each group at different ages. This programme was adhered to except that it was found impossible to label the whole of the plants on one day. All the plants were however labelled at the same stage, *i.e.*, the day before the buds would burst into flower. The plants were most carefully checked and those few plants which, after labelling, did not burst into flower next day, were discarded from the experiment. The age of all the capsules was thus known with certainty to within one day. The table explains the arrangement of the experiments. Two successive lancements were taken from the capsules of each group :—

No. of group	Distinctive group label	No. of plants in group	Date of labelling	DATE OF LANCING		Age of capsules in days from flowering
				First	Second	
1	Red cloth	3,500	11-2-18	17-2-18	19-2-18	6
2	White cloth	3,250	7-2-18	18-2-18	20-2-18	11
3	Paper labels	3,125	12-2-18	28-2-18	2-3-18	16
4	Khaki cloth	3,250	14-2-18	7-3-18	9-3-18	21
5	Black cloth	3,500	15-2-18	13-3-18	15-3-18	26
6	Yellow cloth	3,375	16-2-18	19-3-18	21-3-18	31

To simplify the counting, the field had been divided by means of rope into ten sub-plots. The plants of each group were fairly uniformly distributed over the whole area, in order to rule out as far as possible any effect which might arise owing to want of uniformity of soil, moisture, etc. Further the opium was collected from each group in several samples. The produce of each sample was analysed separately, and the number of plants yielding each sample was separately recorded. In this way an idea could be obtained of the error of the experiment. Moreover, as a further check on the work, on each day on which the plants of a group were first lanced we selected 1,000 capsules which the cultivators considered ready for lancing, and lanced these. Unfortunately, this control was omitted with group 1 and was deliberately omitted with group 4, because the cultivators considered that the capsules of the group lanced that day were at the correct stage for lancing.

*Table showing effect of stage of ripeness of capsules on morphine content of opium produced, 1917-18 experiments.*

			FIRST LANCING					SECOND LANCING				
No. of group	Distinctive label and date of 1st lancing, 2nd lancing two days later	No. of days between flower-ing and 1st lancing	No. of sample	No. of cap-sules produc-ing sample	Per-centage of mor-phine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules gm.	No. of sample	No. of cap-sules produc-ing sample	Per-centage of mor-phine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules gm.
1	Red cloth 17-2-18	6	1	1,500	10.8	65.57	43.7	1	1,476	6.4	14.86	10.1
			2	1,250	11.0	72.98	58.3	2	1,196	6.6	11.80	9.9
			3	870	10.9	47.12	54.2	3	820	5.5	8.78	10.7
				3,620 Total	10.9 Average	185.67 Total	52.1 Average		3,492 Total	6.2 Average	35.44 Total	10.2 Average
2	White cloth 18-2-18	11	1	625	12.7	30.55	48.9	1	612	6.0	14.35	23.0
			2	681	12.6	33.60	49.3	2	671	7.2	11.28	16.0
			3	672	13.4	33.00	49.1	3	695	7.2	12.40	17.8
			4	599	12.7	28.71	47.9	4	588	4.6	7.60	12.9
			5	618	13.2	31.29	50.6	5	601	5.0	8.60	14.3
				3,195 Total	12.9 Average	157.15 Total	49.2 Average		3,167 Total	6.0 Average	54.23 Total	16.9 Average
	18-2-18	Control	1	510	12.4	33.81	66.3	1	511	6.1	11.04	21.6
			2	465	13.3	26.34	56.6	2	470	6.5	8.88	18.9
				975 Total	12.8 Average	60.15 Total	61.5 Average		981 Total	6.3 Average	19.92 Total	20.2 Average



3	Paper labels 28-2-18	16	1 2 3 4	652 741 879 748	12.9 12.5 12.2 13.3	37.21 39.72 48.14 46.38	50.2 53.6 54.8 62.0	1 2 3 4	619 711 902 780	6.1 6.6 6.7 6.8	5.55 6.44 8.8 8.49	9.0 9.0 8.8 11.3
	Control 28-2-18		1 2 3	3,020 698 648 589	12.7 12.0 13.4 13.1	171.45 58.73 53.16 40.01	55.1 84.1 82.0 67.9	1 2 3	3,012 698 648 588	6.5 7.9 7.0 7.4	28.22 8.99 13.46 8.22	9.5 12.8 20.8 14.0
				1,935 Total	12.8 Average	151.90 Total	78.0 Average		1,934 Total	7.4 Average	30.67 Total	15.8 Average
4	Khaki cloth 7-3-18 (No control earned out)	21	1 2 3	1,015 1,041 1,019	13.6 12.3 14.1	45.34 37.00 49.82	44.7 35.6 48.9	1 2 3	910 999 1,003	8.0 6.6 8.3	6.67 3.50 12.8	6.6 3.5 8.49
				3,075 Total	13.3 Average	132.16 Total	43.1 Average		2,912 Total	7.6 Average	20.11 Total	7.4 Average
5	Black cloth 13-3-18	26	1 2 3	1,140 1,085 1,141	14.8 14.7 13.5	33.06 33.66 34.29	29.0 31.0 30.4	1 2 3	1,138 1,052 1,101	9.2 10.1 8.7	6.07 6.18 4.61	5.3 5.7 4.2
	Control 13-3-18		1 2 3	3,366 500 150 350	14.3 14.0 13.9 14.5	101.01 29.05 8.97 21.19	30.1 58.1 59.8 60.5	1 2 3	3,291 500 150 350	9.3 7.1 * }	16.81 Total 3.06	5.1 Average }
				1,000 Total	14.1 Average	59.21 Total	59.2 Average		1,000 Total			
6	Yellow cloth 18-3-18	31										

Capsules over-ripe and yielded no latex.

*Note.*—The number of capsules landed at the second landing does not agree exactly with the number chiefly due to the difficulty of counting so many plants in the field accurately.

*Note*.—The number of capsules landed at the second landing does not agree exactly with the number landed at the first landing. This is chiefly due to the difficulty of counting so many plants in the field accurately.

In reviewing these figures, it appears that the morphine content of the opium of young soft green capsules, such as were used for lancing in group 1, is distinctly lower than that of the opium of riper capsules. When however, as in group 2, the capsules are lanced 11 days after the opening of the flower, the morphine content of the opium is about equal to that from the capsules lanced on the same day at the stage the cultivators considered ready for lancing. Even when the capsules are lanced as in group 5, *i.e.*, 26 days after the flower opens, there is no falling off in the morphine content of the opium, but rather an increase. It is of interest to observe, however, that in the control capsules lanced with groups 2 and 3, the morphine content of the opium is distinctly lower than that of the control capsules lanced with group 5. Moreover, among the groups themselves group 5 has yielded the highest morphine content of opium. The results are shown graphically in Chart II.

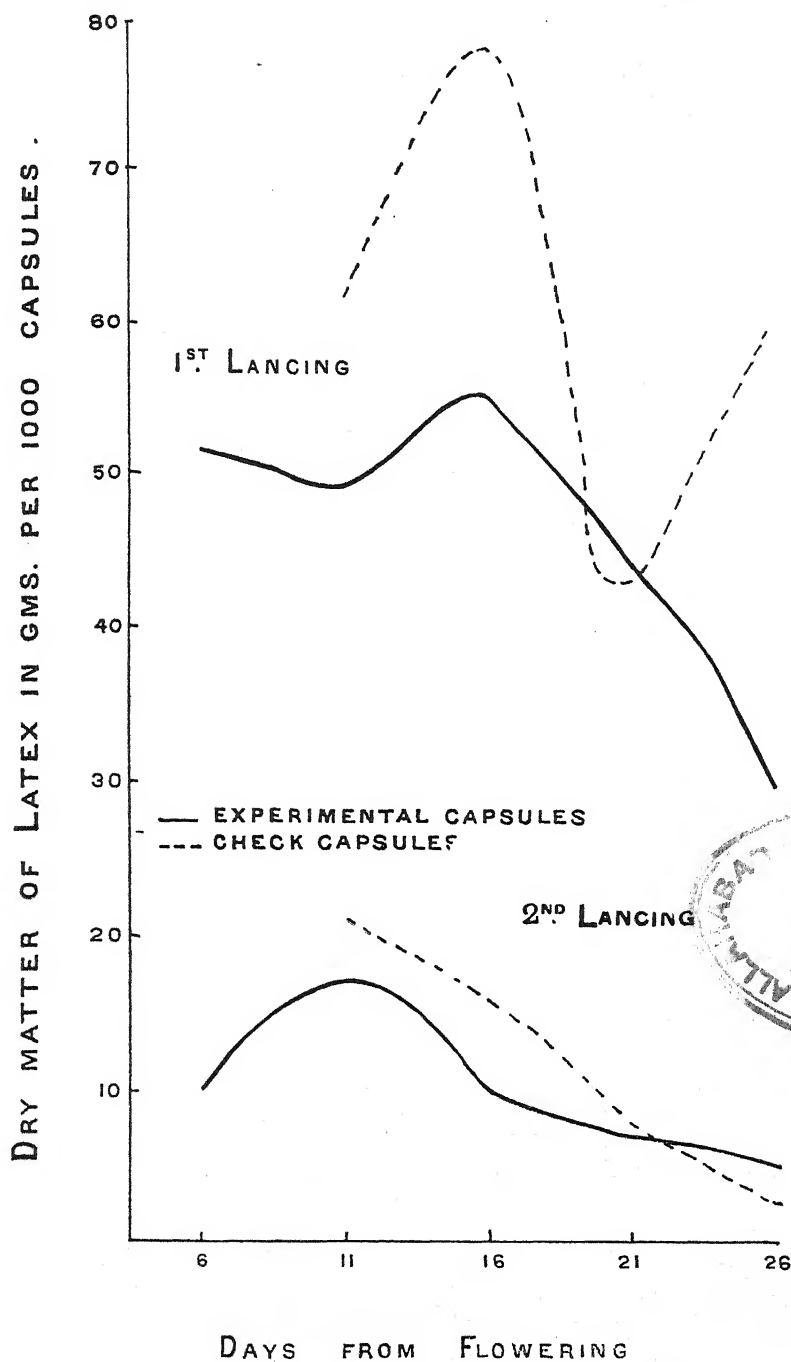
As regards the yield of opium per 1,000 capsules, this has proved more or less constant in the three control series carried out with groups 2, 3, and 5. In the groups, however, it has reached its maximum with group 3, *i.e.*, with capsules lanced 16 days after flowering. With group 5, however (capsules lanced 26 days after flowering), the yield per 1,000 capsules seems to fall off considerably. This is well illustrated in Chart I.

#### 1918-19 EXPERIMENTS.

In the season 1918-19, the experiment was repeated at Cawnpore. The plan of the experiment was the same as in the previous year, but its execution was more satisfactory in that the selected plants were all labelled on the same day at the same stage; moreover, a control lancing of capsules at the correct lancing stage was made on each day on which experimental capsules were lanced and further there were seven groups of plants, the first group being lanced five days from flowering, and each successive group being lanced at four days' intervals. There was an important difference from the previous year's experiment as regards method of lancing adopted. In 1917-18 three vertical incisions were made at each lancing instead of the usual one made by the cultivators. In 1918-19 the ordinary method of a single incision with a 3-bladed knife was used. This difference in lancing should be borne in mind in considering the curves for yield of opium, since, as has been shown<sup>1</sup>, the method of lancing influences the yield of opium. The 1918-19 results are fully set out in the next table and they are illustrated graphically in Charts III and IV.

<sup>1</sup> Annett, Sen, and Singh. *Mem. Dept. Agri. India, Chem. Ser.*, vol. VI, no. 1 (pt. V).

EFFECT OF AGE OF CAPSULES  
ON LATEX YIELD 1917-1918.





EFFECT OF AGE OF CAPSULES ON  
MORPHINE CONTENT OF LATEX 1917-1918.

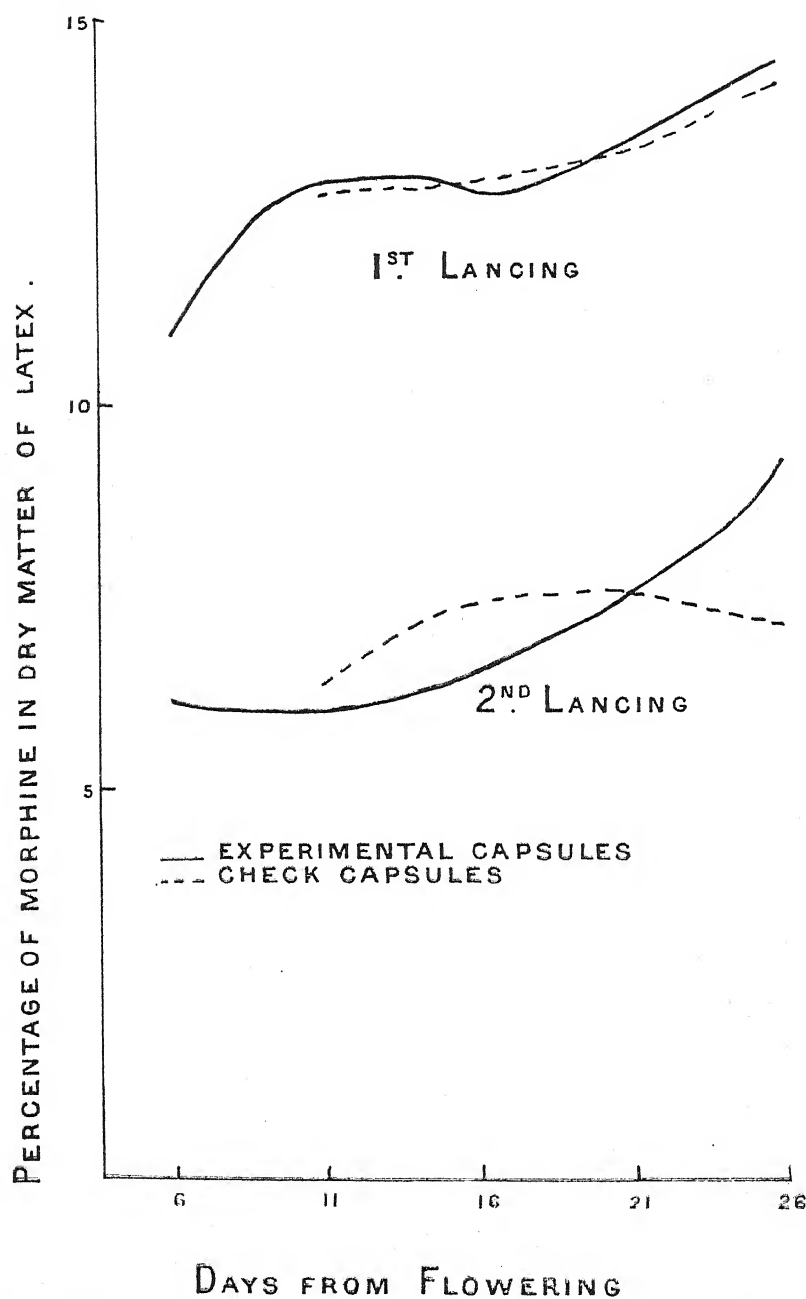






Table shewing effect of stage of ripeness of capsules on morphine content of opium produced, 1918-19 experiments.

No. of group	Distinctive label and date of 1st lancing-2nd lancing two days later	No. of days between flower-ing and 1st lancing	FIRST LANCING					SECOND LANCING				
			No. of sample	No. of capsules producing sample	Percentage of mor-phine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules gm.	No. of sample	No. of capsules producing sample	Percentage of mor-phine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules gm.
1	White labels 22-2-19	5	1	675	9.8	19.30	} 27.70	1	665	6.3	15.49	} 23.05
			2	704	10.6	18.88		2	681	6.7	16.30	
		Control		1,379 Total	10.2 Average	38.18 Total	} 40.36		1,346 Total	6.5 Average	31.79 Total	} 24.72
			1	280	11.2	11.3		1	267	6.3	6.92	
2	Blue labels 26-2-19	9	1	650	11.1	17.28	} 28.24	1	645	8.1	11.82	} 19.69
			2	661	12.2	19.75		2	644	8.6	14.00	
		Control		1,311 Total	11.7 Average	37.03 Total	} 26.01		1,289 Total	8.4 Average	25.82 Total	} 29.51
			1	612	12.3	15.92		1	604	7.2	18.06	
3	Purple labels 2-3-19	13	1	614	11.8	16.46	} 30.40	1	615	8.4	14.53	} 26.08
			2	684	12.5	23.01		2	670	8.3	19.34	
		Control		1,298 Total	12.1 Average	39.47 Total	} 43.08		1,285 Total	8.3 Average	33.87 Total	} 36.07
			1	485	11.9	20.69		1	482	7.9	18.01	
			2	602	11.6	26.15		2	588	7.9	21.20	
				1,087 Total	11.7 Average	46.84 Total			1,070 Total	7.9 Average	39.21 Total	

Table shewing effect of stage of ripeness of capsules on morphine content of opium produced, 1918-19 experiments.

No. of group	Distinctive label and date of 1st lancing, 2nd lancing two days later	No. of days between flowering and 1st lancing	FIRST LANCING						SECOND LANCING					
			No. of sample	No. of capsules producing sample	Percentage of morphine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules	No. of sample	No. of capsules producing sample	Percentage of morphine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules	No. of sample	No. of capsules producing sample
4	Red labels 6-3-19	17	1	509	11.4	17.02	} 36.92	1	488	8.2	10.25	} 21.10		
			2	585	11.5	23.37		2	566	9.2	12.84			
				1,094 Total	11.4 Average	40.39 Total			1,054 Total	8.7 Average	23.09 Total			
			1	519	11.6	22.95		1	516	8.0	13.28			
5	Black labels 10-3-19	Control	2	478	13.0	21.43	} 44.51	2	474	8.4	12.21	} 25.57		
				997 Total	12.3 Average	44.38 Total			990 Total	8.2 Average	25.49 Total			
			1	565	12.1	17.95		1	571	10.4	10.57			
			2	617	12.8	13.14		2	621	11.0	15.59			
	10-3-19	Control		1,182 Total	12.5 Average	31.09 Total	} 42.76		1,192 Total	10.7 Average	26.16 Total	} 42.44		
			1	520	12.4	19.76		1	519	9.9	19.45			
			2	509	13.4	24.22		2	510	10.2	24.21			
				1,029 Total	12.9 Average	43.98 Total			1,029 Total	10.0 Average	43.66 Total			

6	Pink labels 14-3-19	25	1	12.6	9.34	22.14	1	539	8.6	6.65	16.94
				12.4	15.07			556	9.6	12.04	
				12.5	24.41			1,095	9.1	18.69	
				Average	Total			Total	Average	Total	
	14-3-19	Control	1	11.6	21.17	44.27	2	510	8.0	20.69	39.73
				12.7	23.10			490	7.7	19.04	
				12.1	44.27			1,000	7.9	39.73	
				Average	Total			Total	Average	Total	
7	Yellow labels 18-3-19	29	1	12.0	13.57	25.34	2	423	10.4	8.43	8.43
				12.5	11.77			435			
				12.3	25.34			858			
				Average	Total			Total			
	18-3-19	Control	1	10.8	25.73	56.64	2	503	8.2	14.34	30.91
				12.3	30.91			477	8.4	16.57	
				11.6	56.64			980	8.3	30.91	
				Average	Total			Total	Average	Total	

The results obtained appear therefore to confirm those of the previous year.

The curves on Charts I to IV show clearly that as the capsules ripen off their yield of latex rapidly diminishes yet the morphine percentage in the dry matter of the latex does not fall off. One interesting feature of the curves deserves emphasis here. It will be noticed that the morphine content (Charts II and IV) of the opium of the second lancing increases with age of capsule whereas that of the first lancing does not or is certainly marked. Since the yield of opium from the first lancing diminishes rapidly with age of capsule the connection seems established between this fact and the increase in morphine content of the latex from the second lancing with age of capsule. It appears to be indicated that there is a fixed amount of morphine in the capsules, and if the flow of latex at the first lancing is small then the morphine content of a subsequent lancing will be higher than if the first lancing had given a larger yield of latex.

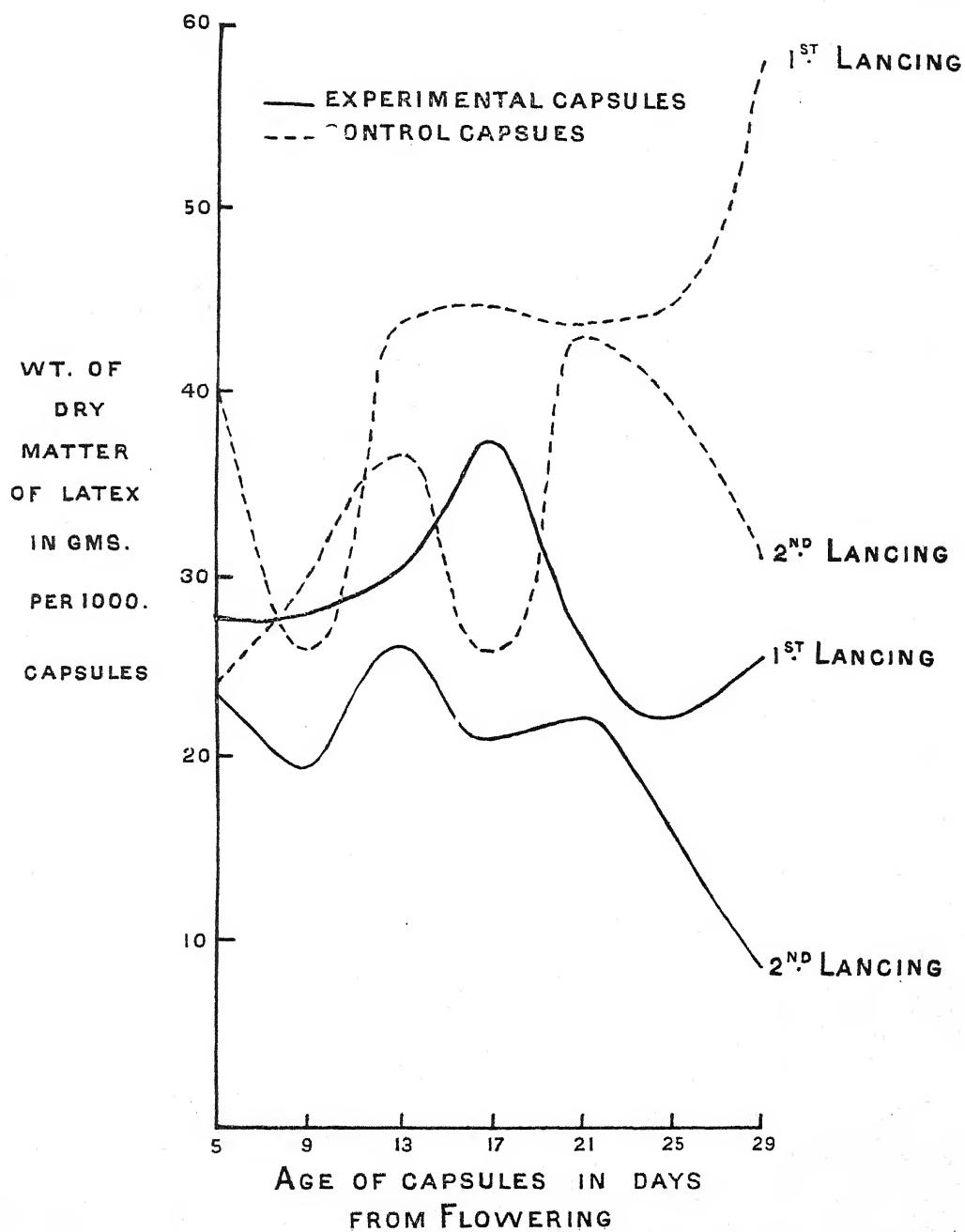
#### 1919-20 EXPERIMENTS.

The experiment was repeated for a third season but on a less ambitious scale. About 3,000 terminal flower buds were selected the day before they were due to burst into flower. They were divided into three groups of about 1,000 in each and the respective groups were labelled with yellow, red and white labels. The capsules of each group were lanced at 5, 16 and 25 days from flowering respectively, and three successive lancements were carried out in each case at three days' intervals. On each of the days when the capsules of our experimental group were lanced, the cultivators were asked to select capsules which they considered ready for lancing. These were lanced with the experimental capsules to provide a check on the experiments and the check capsules also received a second and third lancing. As a rule the produce of each group was collected in more than one portion in order to provide an additional check on the experiment. The table explains the results.

These figures call for little remark since they bear out those of the two previous seasons. It seems perfectly well established that the morphine content of the latex is distinctly lower in the case of very young capsules than it is later on, but the morphine concentration of the latex reaches its maximum before the capsules are actually ripe and thereafter does not again fall off.

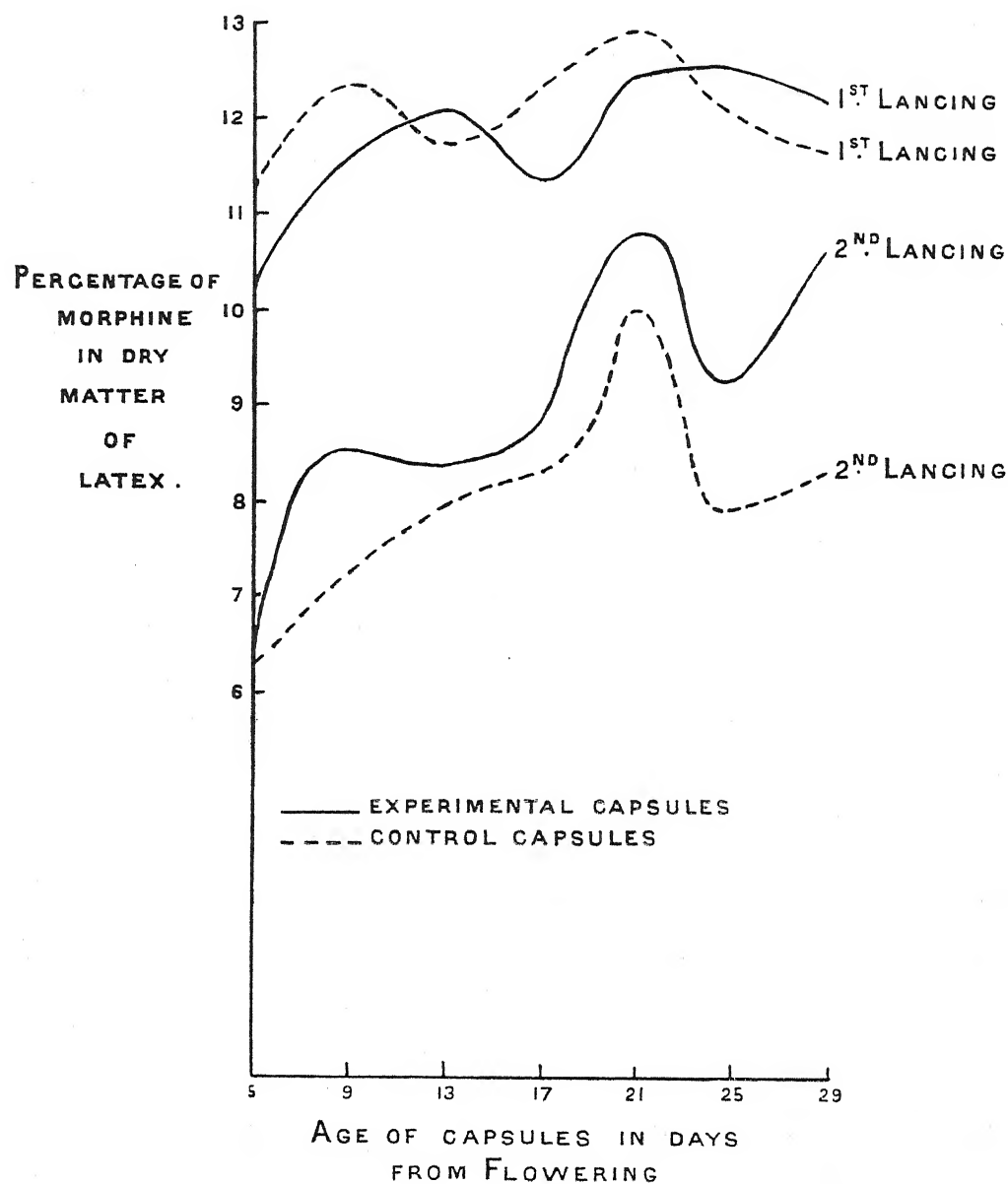


EFFECT OF AGE OF CAPSULES  
ON LATEX YIELD  
1918-1919.





EFFECT OF AGE OF CAPSULE  
ON MORPHINE CONTENT OF LATEX -  
1918-1919.





No. of group	Days between flowering and 1st lancing	No. of lancing	Date of lancing	No. of sample	No. of capsules producing sample	Percentage of morphine in dry op. um	Total grm. dry opium	Dry opium per 1,000 capsules grm.
I	5	First	6-3-20	1	384	11.8	9.84	25.83
				2	327	12.2	10.98	33.58
				3	338	12.7	7.82	23.15
					1,049 Total	12.2 Average	28.64 Total	27.52 Average
		Second	9-3-20	1	388	7.4	5.48	14.29
				2	642	8.2	8.89	12.32
					1,030 Total	7.8 Average	14.37 Total	13.30 Average
		Third	12-3-20	1	377	5.8	9.69	25.82
				2	642	5.1	13.17	19.81
					1,019 Total	5.4 Average	22.86 Total	22.81 Average
	Control	First	6-3-20	1	655	15.0	26.59	40.41
				2	411	16.2	17.18	41.81
				3	400	15.4	14.32	35.79
					1,466 Total	15.5 Average	58.09 Total	39.34 Average
		Second	9-3-20	1	648	11.2	21.43	32.72
				2	411	12.6	13.16	32.03
				3	393	12.0	12.41	31.02
					1,452 Total	11.9 Average	47.00 Total	31.92 Average
		Third	12-3-20	1	632	6.8	12.38	18.91
				2	406	7.3	15.58	37.90
				3	399	6.8	14.68	36.67
					1,437 Total	7.0 Average	42.64 Total	31.16 Average



No. of group	Days between flowering and 1st lancing	No. of lancing	Date of lancing	No. of sample	No. of capsules producing sample	Percentage of morphine in dry opium	Total gm. dry opium	Dry opium per 1,000 capsules gm.
II	16	First	17-3-20	1	458	14.0	8.96	19.56
				2	534	14.6	15.92	29.82
					992 Total	14.3 Average	24.88 Total	24.69 Average
		Second	20-3-20	1	420	11.4	5.91	12.89
				2	512	11.2	8.88	16.63
					932 Total	11.3 Average	14.79 Total	14.76 Average
		Third	23-3-20	1	878	6.4	3.71	3.74
	Control	First	17-3-20	1	600	14.6	25.34	42.23
				2	500	14.6	25.63	51.26
					1,100 Total	14.6 Average	50.97 Total	46.74 Average
		Second	20-3-20	1	500	10.3	12.96	25.92
		Third	23-3-20	1	406	5.0	4.27	8.54
*III	25	First	26-3-20	1	518	16.3	5.79	11.19
	Control	First	26-3-20	1	500	15.1	14.79	29.58

\* Note. Heads dried up after 1st lancing.

#### ( CONCLUSIONS.

1. Very soft immature capsules, say six days old from the date of flowering, produce opium of lower morphine content than older capsules.

2. Provided they are not less than about eight days old from the date of flowering, the age of the capsule has but little effect on the percentage of morphine in the opium produced.

The probability is, therefore, that as the capsules ripen their morphine content does not decrease as has been stated by other workers.

3. As regards yield of opium per capsule this reached a maximum in our experiments when the capsules were 16-17 days old from the date of flowering. Subsequently the yield of opium per capsule diminished.

#### THE INFLUENCE OF MANURES.

A review of the literature shows that there is a fairly widespread opinion that the alkaloidal content of plants can be affected by manuring.

Henry<sup>1</sup> states that "the quantity of alkaloid can be greatly increased by special cultivation."

Tunmann<sup>2</sup> states that "the alkaloid content can be increased by suitable manuring, but this effect is only indirect since more robust plants are produced." In this connection he mentions the names of Vanderlinden, Feldhaus, Chevalier and Mitlacher.

Work by Wayne Army<sup>3</sup> on belladonna shows, however, that small plants contained the highest percentage of atropine. It has been stated also that, in the case of indigo, stunted plants contain the highest amounts of the glucoside indican. These facts would appear to lead one to expect bigger plants from manuring with consequent lower percentages of alkaloids in those plants.

Carr<sup>4</sup> and Reynolds refer to manurial experiments on belladonna and henbane. The results are tabulated below:—

Manures	PERCENTAGE OF TOTAL ALKALOIDS	
	Belladonna	Henbane
Unmanured . . .	0.66	0.20
Kainit . . .	0.61	0.13
Superphosphate . . .	0.46	0.11
Basic slag . . .	0.60	0.13
Farmyard manure . . .	0.55	....
Sodium nitrate . . .	0.52	....

It does not seem that any definite conclusions can be drawn from these experiments.

Carr<sup>5</sup> has published the results of experiments carried on at Dartford, Kent, during six seasons on the effect of manures on the alkaloidal content of

<sup>1</sup> Plant Alkaloids, p. 10.

<sup>2</sup> *Pflanzen microchemie*, p. 264.

<sup>3</sup> "Breeding for atropine." *Journ. of Heredity*, vol. III, April 1917.

<sup>4</sup> *Pharm. Journal*, IV, 26, 543.

<sup>5</sup> *Amer. J. of Pharmacy*, vol. 85, no. 11, 1913, p. 487, and *Eighth International Congress of Applied Chem.*, vol. XVII, p. 7.

belladonna. He states that the land used was naturally suited to the plants and the percentage of alkaloids obtained without manure was already high. The manures employed were farmyard manure, sodium nitrate, calcium cyanamide, basic slag, superphosphate and kainit. He concluded that the effects of fertilizers upon the percentage of alkaloid is small, tending to lower it. As regards the amount of plant harvested he states that a rich nitrogenous fertilizer will increase it severalfold if the soil is not already a rich one. Carr's experiments in this direction seem to be the most complete so far published. As will be seen later, they closely support our own conclusions in the case of opium.

Chevalier<sup>1</sup> has published results purporting to show that the use of nitrates with farmyard manure doubles the alkaloidal content of the dried leaves of belladonna, hyoscyamus, and stramonium, and he also concludes that potash and phosphates do not affect the alkaloidal content. His results have been referred to as conclusive<sup>2</sup> but the short account of his work appearing in the *Comptes rendus* would not seem to afford sufficient grounds for this opinion. The following is a reproduction of his tabulated results:—

Treatment	Total alkaloids in 100 gm. dried leaves gm.
Fields under ordinary cultivation and manuring .. ..	0.320 0.336
Fields receiving phosphoric acid and potash .. ..	0.480 0.490
Garden receiving nitrogenous manures but neither } 2 year old plants phosphoric acid nor potash .. .. } Young plants ..	0.616 0.406
Fields receiving nitrogenous manures .. ..	
(a) Strong soil .. ..	0.676
(b) Vineland-pebbly soil .. ..	0.680
(c) Light land with dung and nitrate added .. ..	0.756

It is apparently from the above figures that Chevalier has drawn his conclusions. Speaking from considerable experience with manurial experiments the writer would certainly not draw such general conclusions without much more supporting data. In the first place in work of this nature the error of experiment must be very large and this error does not seem to have

<sup>1</sup> *Comptes rendus*, 1910, 150, p. 344.

<sup>2</sup> *Year Book of Pharmacy*, 1910. Presidential Address to Brit. Pharm. Conf., p. 314. Also Henry, *Plant Alkaloids*, p. 10.

been ascertained. Moreover, all the experiments have not been carried out on the same type of land and therefore it is hardly fair to compare the results. It would also be interesting to know whether or not the same pure race of plants was used in the tests. Unless it was, this fact would also detract considerably from the value of the results.

Ransom<sup>1</sup> and Henderson carried out experiments with various manures on belladonna at Hitchin. The results brought no very definite conclusion and certainly do not lend much support to Chevalier's view that nitrate encourages alkaloid production. The unmanured plot produced plant with the highest alkaloid content. Rasmussen<sup>2</sup>, in studies on the protein and nicotine content of tobacco plants during growth, found no clear relation between the fertilizers used and the nicotine contents of the resulting plants. The total nitrogen varied during growth according to the variety of the plant and the situation of the leaves tested.

Mitlacher<sup>3</sup> and Hayes report that manuring with stable or artificial manure does not have much influence on good soil on alkaloidal production in opium but state that on a poor soil the influence is marked.

Mukerji<sup>4</sup> in experiments at Pusa, India, is unable to trace any connection between manurial treatment and the nicotine content of tobacco leaves.

In short, the literature of the subject provides little satisfactory evidence that the alkaloidal content of drug-yielding plants can be modified by manuring.

The writer has carried out at Cawnpore during four seasons a careful series of experiments in order to see if any particular system of manuring of poppy has any influence on the alkaloidal content of the opium produced. The results are set out below.

#### 1916-17 EXPERIMENTS.

Two quite separate series of experiments were carried out on land which had been previously unmanured, except in the case of series II, the land used for that having received farmyard manure in 1913 at the rate of  $5\frac{1}{2}$  tons per acre. It was intended to use a different pure race of poppy, isolated by Mr. H. M. Leake, Economic Botanist to Government, United Provinces, for each series. Unfortunately in one series (series I) the seed used had been accidentally mixed with the seed of another race, and the resulting crop was a

<sup>1</sup> *International Congress of Applied Chemistry, N. Y.*, 1913. Through *Chem. and Drugg.*, 1912, 81,443.

<sup>2</sup> *Biochem. Ztschr.* 69 (1915), No. 5-6, pp. 461 to 466. Through *Exp. Station, Record*, Sept. 1916, p. 333.

<sup>3</sup> *Year Book of Pharmacy*, 1914, p. 201.

<sup>4</sup> *Sc. Rep. Agr. Res. Inst., Pusa*, p. 30, 1917-18.

mixture. The results in this case are therefore not so valuable as those from the other series (series II), in which no mistake occurred with the seed. Series I was carried out in field No. 29. The total area of this field was about 0.5 acre. It was divided into 16 plots, each 81' by 13'; and all around each sub-plot was a margin of crop 2' wide which was excluded from the experiment.

Series II was carried out on field No. 9 and in a similar manner to series I. The total area of the field was about one-third of an acre. The 16 plots each measured 45' by 18'. The crop in each case was sown on 18th November 1916 after having been irrigated on 13th November. The seed rate used was 6 lb. per acre.

*Manuring.* The diagram and table show the arrangement of the plots, which was similar in each series.

*Diagram showing arrangement of manurial plots.*

1	16
2	15
3	14
4	13
5	12
6	11
7	10
8	9

*Table showing treatment of manurial plots.*

<i>Plot No.</i>	<i>Treatment</i>
1 and 11 .. ..	Superphosphate and nitrate of soda.
2 and 9 .. ..	Superphosphate, potassium sulphate and nitrate of soda.
3 and 12 .. ..	Potassium sulphate and nitrate of soda.
4 and 10 .. ..	Potassium sulphate and superphosphate.
5 and 15 .. ..	Nitrate of soda.
6 and 13 .. ..	Superphosphate.
7 and 16 .. ..	Potassium sulphate.
8 and 14 .. ..	Unmanured.

Each plot therefore had a duplicate in each series.



*Amounts of manures and time of application.*

IN series I, field No. 29, superphosphate and potassium sulphate were applied at the rate of 400 lb. per acre each. The superphosphate and potassium sulphate were applied on the same day as and just before the seed. The nitrate of soda was put on in two equal dressings (at the rate of 120 lb. per acre each), the first on 2nd January 1917 and the second on 21st January 1917. The land was irrigated on the day following each application.

In series II, field No. 9, exactly the same procedure was followed, except that by an oversight the superphosphate and potassium sulphate were applied at the rate of only 320 lb. per acre.

*Observations during growth.*

The plots receiving potash alone showed no improvement over the unmanured ones. Wherever superphosphate had been applied the effect was very marked in a more highly developed plant but no connection could be observed between time of flowering and any particular manurial application. The effect of the nitrate was very marked indeed, and the plots to which it had been applied could be picked out within a few days owing to the dark green colour of their foliage. The nitrate plots seemed subsequently, markedly the best plots of all.

*Harvesting of produce.*

*Opium.* The capsules were lanced with a 4-bladed knife<sup>1</sup>. Each of the plots on field 9 received seven lancements carried out on the 8th, 11th, 14th, 17th, 20th, 23rd and 26th March, respectively. Each of the plots on field 29 received six lancements, which were carried out on the 8th, 11th, 14th, 17th, 20th and 23rd March, respectively.

The product of each lancing was kept separate. This is very necessary, since the opium of the first lancing is much the richest in morphine, and the amount of this alkaloid falls off rapidly and progressively in the opium from each of the succeeding lancements<sup>2</sup>. The total dry weight and the morphine content of the opium obtained at each lancing were estimated.

*Straw and capsules.* After lancing, the crop was left till dead ripe. The hot sun had by this time practically dried up all green parts of the plant. The total air dry weight of the plant on each plot was then recorded and also the total air dry weight of the capsules. The number of plants, and also the number of capsules on each plant, was also counted. This was done in order

<sup>1</sup> For details of method by which opium is extracted from the capsule in India see *Mem. Dept. Agri. India (Chem. Series)*, vol. VI, no. 1 (pt. II).

<sup>2</sup> *Loc. cit.*, part III.

to see if the number of capsules borne per plant had any connection with the manure used. This is an important point, because it has been shown that the oldest capsule produces more opium, and that of a higher morphine content, than the subsequent capsules appearing on the same plant. (See p. 74.)

The results obtained are summarized in the Tables I to VII and illustrated diagrammatically in Chart V.

TABLE I.

*Effect of manures on morphine content of opium.*

No. of plot	Manurial treatment	PER CENT. OF MORPHINE IN OPIUM DRIED AT 100°C.						
		1st lanc-ing	2nd lanc-ing	3rd lanc-ing	4th lanc-ing	5th lanc-ing	6th lanc-ing	7th lanc-ing
Field No. 29.								
1	Super + NaNO <sub>3</sub>	10.0	7.7	3.6	3.5	2.7		
2	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	10.2	8.1	3.9	2.9	2.6		
3	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	9.5	9.1	5.0	4.1	..		
4	K <sub>2</sub> SO <sub>4</sub> + super	9.7	..	4.9	3.9	3.1		
5	NaNO <sub>3</sub>	9.1	7.9	4.3	5.9	2.7		
6	Superphosphate	9.3	7.1	5.8	3.8	2.6	2.3	
7	K <sub>2</sub> SO <sub>4</sub>	8.6	6.6	5.7	4.1	2.3		
8	No manure	7.6	8.0	3.7	4.1	2.3		
9	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	9.0	4.9	4.3	4.0	1.5		
10	K <sub>2</sub> SO <sub>4</sub> + super	9.6	6.5	4.4	1.4	..		
11	Super + NaNO <sub>3</sub>	9.8	7.6	5.2	4.7	1.9		
12	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	9.8	7.6	4.4	4.3	2.0		
13	Superphosphate	10.3	7.9	5.3	3.0	1.2		
14	No manure	9.6	8.1	3.5	3.3	..		
15	NaNO	11.0	6.9	3.4	3.5	..		
16	K <sub>2</sub> SO	9.7	7.3	3.8	3.7	..		
Field No. 9.								
1	Super + NaNO <sub>3</sub>	12.0	9.8	6.5	..	1.6	1.8	
2	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	11.6	7.8	5.4	..	1.6	1.4	
3	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	10.2	8.7	4.8	..	2.0	..	
4	Super + K <sub>2</sub> SO <sub>4</sub>	9.1	8.7	4.3	..	2.0	..	
5	NaNO <sub>3</sub>	10.7	8.8	6.4	4.8	3.3	2.0	
6	Superphosphate	11.3	9.2	3.6	4.0	2.5	1.2	
7	K <sub>2</sub> SO <sub>4</sub>	9.6	8.7	5.6	5.3	2.3	1.9	
8	No manure	8.9	8.0	5.5	..	2.5	1.6	
9	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	10.8	9.9	5.0	..	3.6	2.4	
10	K <sub>2</sub> SO <sub>4</sub> + super	10.3	9.6	7.1	..	3.9	1.3	
11	Super + NaNO <sub>3</sub>	10.6	10.7	7.3	..	2.8	2.4	
12	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	11.4	10.0	7.9	..	3.7	2.0	
13	Superphosphate	10.3	9.6	4.4	..	1.9	2.3	
14	No manure	9.9	8.3	5.0	..	1.3	..	
15	NaNO <sub>3</sub>	11.0	9.6	6.2	4.0	1.9	1.3	
16	K <sub>2</sub> SO <sub>4</sub>	9.7	8.8	6.0	..	1.4	1.2	

TABLE II.

*Effect of manures on out-turn of opium.*

No. of plot	Manurial treatment	WEIGHT OF OPIUM DRIED AT 100°C. IN GRM.							Total weight of opium per plot dried at 100°C. grm.	Opium dried at 100°C. lb. per acre
		1st lanc-ing	2nd lanc-ing	3rd lanc-ing	4th lanc-ing	5th lanc-ing	6th lanc-ing	7th lanc-ing		
Field No. 29.										
1	Super + NaNO <sub>3</sub>	82.05	91.31	31.43	15.05	4.60	0.94	..	225.47	20.59
2	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	75.49	75.06	55.43	17.59	7.82	2.00	..	233.39	21.31
3	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	42.54	65.05	49.84	16.50	3.82	1.49	..	179.24	16.36
4	Super + K <sub>2</sub> SO <sub>4</sub>	29.39	27.53	42.54	13.28	6.12	2.88	..	121.74	11.11
5	NaNO <sub>3</sub>	18.97	67.12	52.30	25.82	8.14	1.96	..	174.33	15.92
6	Superphosphate	37.25	34.65	38.56	20.33	6.60	4.29	..	141.68	12.93
7	K <sub>2</sub> SO <sub>4</sub>	27.52	29.57	40.51	15.27	4.21	2.04	..	119.12	10.87
8	No manure	15.20	35.87	36.28	24.66	5.32	3.62	..	120.95	11.05
9	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	67.08	63.40	37.82	15.18	4.46	2.30	..	190.24	17.37
10	Super + K <sub>2</sub> SO <sub>4</sub>	47.33	51.92	42.63	20.37	6.98	3.34	..	172.57	15.76
11	Super + NaNO <sub>3</sub>	79.45	69.96	61.72	29.88	8.66	2.74	..	252.41	23.04
12	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	52.15	69.52	52.27	28.83	5.82	1.12	..	209.71	19.14
13	Superphosphate	46.49	43.65	37.95	15.70	4.03	3.38	..	151.20	13.80
14	No manure	46.35	55.92	40.03	13.26	2.55	0.85	..	158.96	14.52
15	NaNO <sub>3</sub>	83.25	66.62	47.73	13.15	4.00	2.49	..	217.24	19.89
16	K <sub>2</sub> SO <sub>4</sub>	51.15	47.86	35.10	14.63	2.68	0.21	..	151.63	13.85
Field No. 9.										
1	Super + NaNO <sub>3</sub>	24.33	54.35	42.81	14.50	9.95	5.51	2.19	153.64	17.99
2	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	41.25	61.91	34.93	12.30	7.85	4.69	1.86	164.79	19.30
3	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	25.60	57.19	32.48	13.50	7.64	1.98	1.60	139.99	16.39
4	Super + K <sub>2</sub> SO <sub>4</sub>	19.55	37.13	30.03	10.80	4.66	2.00	0.99	105.16	12.31
5	NaNO <sub>3</sub>	20.17	38.12	38.60	24.85	9.50	5.46	2.17	138.87	16.27
6	Superphosphate	25.57	35.02	32.70	17.00	10.91	4.61	3.49	129.30	15.14
7	K <sub>2</sub> SO <sub>4</sub>	8.88	28.96	33.44	19.05	8.36	4.60	3.52	106.81	12.50
8	No manure	16.11	27.40	31.20	13.30	8.73	5.43	2.38	104.55	12.25
9	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	41.58	41.50	49.40	29.00	32.22	13.34	6.82	213.86	25.04
10	Super + K <sub>2</sub> SO <sub>4</sub>	20.50	30.06	34.22	30.30	16.02	7.56	5.21	143.87	16.85
11	Super + NaNO <sub>3</sub>	35.80	49.55	44.20	24.60	12.45	10.57	8.22	185.39	21.71
12	NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	27.11	44.15	49.05	30.50	13.57	8.92	3.55	176.85	20.71
13	Superphosphate	13.98	32.13	32.13	32.37	10.71	4.63	1.89	118.41	13.87
14	No manure	18.55	32.34	23.20	14.00	5.70	1.47	0.38	95.64	11.19
15	NaNO <sub>3</sub>	27.90	56.86	40.16	19.90	7.63	7.00	2.54	161.99	18.97
16	K <sub>2</sub> SO <sub>4</sub>	15.71	40.78	32.73	18.50	6.54	4.31	1.60	120.17	14.07

In the following table the yield of opium is expressed as the quantity yielded per 1,000 capsules.

TABLE III.

*Effect of manure on out-turn of opium.*

No. of plot	Manurial treatment	No. of capsules per plot	No. of capsules per plot ; average of duplicates	Grm. dried opium per 1 000 capsules	Average of duplicates previous column	Weight of dried opium per 1 000 capsules unmanured plot = 100
<i>Field No. 29.</i>						
8	Unmanured . . . . .	4,487	4,290	27.0	32.9	100
14	" . . . . .	4,094		38.9		
7	K <sub>2</sub> SO <sub>4</sub> . . . . .	5,015	4,762	23.8	28.7	87
16	" . . . . .	4,510		33.6		
6	Superphosphate . . . . .	3,895	4,049	36.4	36.2	110
13	" . . . . .	4,203		36.0		
5	NaNO <sub>3</sub> . . . . .	3,857	4,227	45.2	46.2	140
15	" . . . . .	4,598		47.2		
4	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	3,540	3,818	34.4	38.2	116
10	" . . . . .	4,086		42.1		
3	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	4,804	4,080	37.3	49.9	152
12	" . . . . .	3,357		62.5		
1	Super + NaNO <sub>3</sub> . . . . .	4,291	4,225	52.6	56.6	172
11	" . . . . .	4,159		60.7		
2	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub> . . . . .	4,172	3,964	56.0	53.3	162
9	" . . . . .	3,756		50.6		

*Field No. 9.*

8	Unmanured . . . . .	1,897	2,145	55.1	47.5	100
14	" . . . . .	2,394		40.0		
7	K <sub>2</sub> SO <sub>4</sub> . . . . .	2,296	2,511	46.5	45.3	96
16	" . . . . .	2,726		44.1		
6	Superphosphate . . . . .	2,718	2,726	47.6	45.4	96
13	" . . . . .	2,734		43.3		
5	NaNO <sub>3</sub> . . . . .	2,425	2,527	57.3	59.3	125
15	" . . . . .	2,629		61.3		
4	K <sub>2</sub> SO <sub>4</sub> + Super . . . . .	2,578	2,671	40.8	46.4	98
10	" . . . . .	2,765		52.0		
3	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	2,375	2,703	59.0	58.6	123
12	" . . . . .	3,032		58.3		
1	Super + NaNO <sub>3</sub> . . . . .	2,755	2,772	55.8	62.3	131
11	" . . . . .	2,690		68.9		
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	2,366	2,470	69.7	76.4	161
9	" . . . . .	2,574		83.1		

TABLE IV

*Shewing relation between yield of opium per plot and per 1,000 capsules.*

No. of plot	Manurial treatment	Total weight of opium dried at 100°C. per plot gram.	Average of duplicates previous column	Yield of opium per plot average of unmanured = 100	Grm. opium per 1,000 capsules	Average of duplicates previous column	Yield per 1,000 capsules average of unmanured = 100
<i>Field No. 29.</i>							
8 14	Unmanured . . .	120.9 } 150.9 }	139.9	100	27.0 } 38.9 }	32.0	100
7 16	K <sub>2</sub> SO <sub>4</sub> . . .	119.1 } 151.6 }	135.3	97	23.8 } 33.6 }	28.7	87
6 13	Superphosphate . .	141.7 } 151.2 }	146.4	105	36.4 } 36.0 }	36.2	110
5 15	NaNO <sub>3</sub> . . .	174.3 } 217.2 }	195.7	140	45.2 } 47.2 }	46.2	140
4 10	K <sub>2</sub> SO <sub>4</sub> + super . .	121.7 } 172.6 }	147.1	105	34.4 } 42.1 }	38.2	116
3 12	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . .	179.2 } 209.9 }	194.5	139	37.3 } 62.5 }	49.9	152
1 11	Super + NaNO <sub>3</sub> . .	225.5 } 252.4 }	235.9	171	52.6 } 60.7 }	56.6	172
2 9	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	233.4 } 190.2 }	211.8	151	56.0 } 50.6 }	53.3	162
<i>Field No. 9.</i>							
8 14	Unmanured . . .	104.5 } 95.6 }	100.0	100	55.1 } 40.0 }	47.5	100
7 16	K <sub>2</sub> SO <sub>4</sub> . . .	106.8 } 120.2 }	113.5	113	46.5 } 44.1 }	45.3	96
6 13	Superphosphate . .	129.3 } 118.4 }	123.8	124	47.6 } 43.3 }	45.4	96
5 15	NaNO <sub>3</sub> . . .	138.9 } 162.0 }	150.4	150	57.3 } 61.3 }	59.3	125
4 10	K <sub>2</sub> SO <sub>4</sub> + super . .	105.2 } 143.9 }	124.5	124	40.8 } 52.0 }	46.4	98
3 12	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . .	140.0 } 176.8 }	158.4	158	59.0 } 58.3 }	58.6	123
2 11	Super + NaNO <sub>3</sub> . .	153.6 } 185.4 }	169.5	169	55.8 } 68.9 }	62.3	131
2 9	Super + NaNO <sub>3</sub> + K <sub>2</sub> SO <sub>4</sub>	164.8 } 213.9 }	189.3	189	69.7 } 83.1 }	76.4	161



TABLE V

*Shewing effect of manure on number of plants and capsules per plot.*

No. of plot	Manurial treatment	No. of plants	Average of duplicates previous column	No. of plants per plot average of unmanured plots = 100	No. of capsules	Average of duplicates previous column	No. of capsules per plot average of unmanured = 100	Capsules per 100 plants
Field No. 29.								
8	Unmanured . . . .	4,354	4,082	100	4,487	4,290	100	105
14		3,811			4,094			
7		4,939			5,015			
16	K <sub>2</sub> SO <sub>4</sub> . . . .	4,158	4,548	111	4,510	4,762	110	105
6		3,634			3,895			
13		3,407			4,203			
5	Superphosphate . . . .	3,572	3,520	86	3,857	4,049	94	115
15		4,240			4,598			
4		3,341			3,540			
10	K <sub>2</sub> SO <sub>4</sub> + super . . . .	3,667	3,454	85	4,096	3,818	89	110
3		4,555			4,808			
12		2,991			3,357			
1	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	3,953	3,773	92	4,291	4,080	95	108
11		3,685			4,159			
2		3,697			4,172			
9	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	3,079	3,388	83	3,756	3,964	92	117
Field No. 9.								
8	Unmanured . . . .	1,822	2,094	100	1,897	2,145	100	102
14		2,366			2,394			
7		2,235			2,296			
16	K <sub>2</sub> SO <sub>4</sub> . . . .	2,269	2,452	117	2,726	2,511	117	102
6		2,606			2,718			
13		2,607			2,734			
5	Superphosphate . . . .	2,332	2,606	124	2,425	2,726	127	105
15		2,468			2,629			
4		2,531			2,578			
10	K <sub>2</sub> SO <sub>4</sub> + Super . . . .	2,609	2,570	123	2,765	2,671	125	104
3		2,265			2,765			
12		2,944			3,032			
1	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	2,619	2,604	124	2,755	2,703	126	104
11		2,568			2,690			
2		2,216			2,366			
9	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	2,008	2,112	101	2,574	2,470	115	117

## CONTROL EXPERIMENT.

In addition a control experiment was carried out in order to determine the variations in opium yield and of morphine content of the crop from different portions of the same field, all of which had received uniform treatment. Such an experiment is of prime importance in enabling one to decide whether any different results obtained in various manurial plots are really due to a particular manurial treatment or not. For the purpose of this control experiment of

course a pure race of poppy was used—one isolated by Mr. H. M. Leake. The field contained eight plots, each measuring 34·25' × 18·4', and each plot was surrounded by a margin of non-experimental crop, 2½ feet wide. Five successive lancements were made on each plot and the results are set out below :—

TABLE VI.

No. of plot	PER CENT. OF MORPHINE IN OPIUM DRIED AT 100°C. IN EACH LANCING				
	1st	2nd	3rd	4th	5th
1	11·6	10·9	11·9	7·4	6·7
2	14·3	11·5	11·5	8·5	6·7
3	13·1	10·9	10·4	6·4	5·2
4	14·3	..	..	..	..
5	12·6	10·6	8·9	4·2	4·3
6	11·5	10·0	8·3	5·9	..
7	13·1	10·5	7·6	5·0	4·8
8	11·9	11·3	8·4	5·2	..

TABLE VII.

No. of plot	WT. OF OPIUM DRIED AT 100°C. FROM EACH LANCING. GRM.					Total weight of opium per plot grm.	Lb. of opium per acre	No. of cap- sules per plot	Grm. of opium per 1,000 cap- sules
	1st	2nd	3rd	4th	5th				
1	22·22	21·41	19·83	19·17	8·86	91·49	13·96	2,371	30·6
2	9·00	18·87	27·65	31·87	11·14	98·53	15·03	2,415	40·8
3	19·88	22·35	24·35	27·67	7·32	101·57	15·50	2,294	44·3
4	15·38	20·74	22·68	34·00	8·75	101·55	15·50	2,238	45·4
5	18·49	32·71	17·55	10·72	5·32	84·79	12·94	2,478	34·2
6	12·77	23·54	13·80	10·03	3·06	63·20	9·64	2,267	27·9
7	19·22	23·25	11·88	11·25	4·04	69·64	10·63	2,075	33·5
8	15·14	23·24	14·42	13·54	2·91	69·25	10·56	2,124	32·6

## CONCLUSIONS DRAWN FROM 1916-17 EXPERIMENTS.

A. *Effect of manures on out-turn of opium.*

Table II gives very definite information on this point, though the results are perhaps set out in a more convenient form in Table IV, col. 5. *Nitrate of soda* has had a very marked effect in increasing the out-turn of opium in every case. *Sulphate of potash* seems to have had no effect whatever, either alone or in combination with nitrate of soda or superphosphate. *Superphosphate* certainly seems to have had some slight effect in increasing the yield of opium. The crop in its young stages showed to the eye a marked beneficial effect in every case from the application of superphosphate, but this effect passed away as the crop grew bigger.

On field No. 9 superphosphate alone appears to have given a 24 per cent. increase in opium yield, as on both fields superphosphate and nitrate of soda gave a distinct increase over the nitrate of soda plot, and also over the plot receiving sulphate of potash plus nitrate of soda. Further Table IV shows that plots receiving superphosphate together with potash and nitrate of soda have given distinctly more opium than the plot to which only the potash and nitrate of soda were applied.

The outstanding feature, however, is the beneficial effect of the nitrate of soda.

It will be of interest to see in what way this increase of out-turn has been brought about. It might be brought about either by the manure increasing the number of capsules in a given area or by increasing the yield of opium per capsule. Table III gives a column showing the number of capsules in each manurial plot. From this it will readily be seen that the increase in yield is not attributable to the production of a larger number of capsules. Moreover let us take an instance on field 29. The unmanured plot No. 8 had 4,487 capsules. The nitrate of soda plot No. 5 had 3,857 capsules, yet the former only gave 11.05 lb. dry opium per acre as against 15.92 lb. yielded by the latter. It would seem obvious that the increase in yield brought about by nitrate of soda was due to an increase in yield of the individual capsules.

Table IV shows the relation between the yield of opium per plot and per 1,000 capsules in each plot. A comparison between cols. 5 and 8 of that table is interesting, for the relation between the yield per plot, and yield per 1,000 capsules in each plot, is distinct and especially so in field 29.

In the field one could not help noticing how much bigger the capsules on the nitrate of soda plots were than those on plots receiving no nitrate of soda.

*B. Effect of manure on morphine content of the opium.*

In considering this effect one should only take into consideration the morphine content of the first lancements,<sup>1</sup> since at the second lancing the opium obtained is a mixture of the produce of heads some of which have been lanced twice and others only once. The morphine content of the opium of the later lancements has been given as a matter of interest.

A study of Table I shows that on field 29 the morphine content of the opium of the first lancements has varied from 7.6 to 11.0 and in field 9 from 8.9 to 12.0. Before considering these variations it is of importance to refer to Table VI which gives the morphine content of the opium grown on 8 plots of an unmanured field. It will be seen that the morphine content varied from 11.5 to 14.3 per cent. on different parts of the field. This is about the range of the variation in morphine content of opium of the first lancing which we have since found in experiments in which we have grown five or six acres of the same pure race of poppy. It is almost as great as the range of variation in morphine content on the various plots of fields 9 and 29. Hence the effect of manure on the morphine content cannot be very marked.

The following table will help in considering any possible manurial effect :—

TABLE VIII.

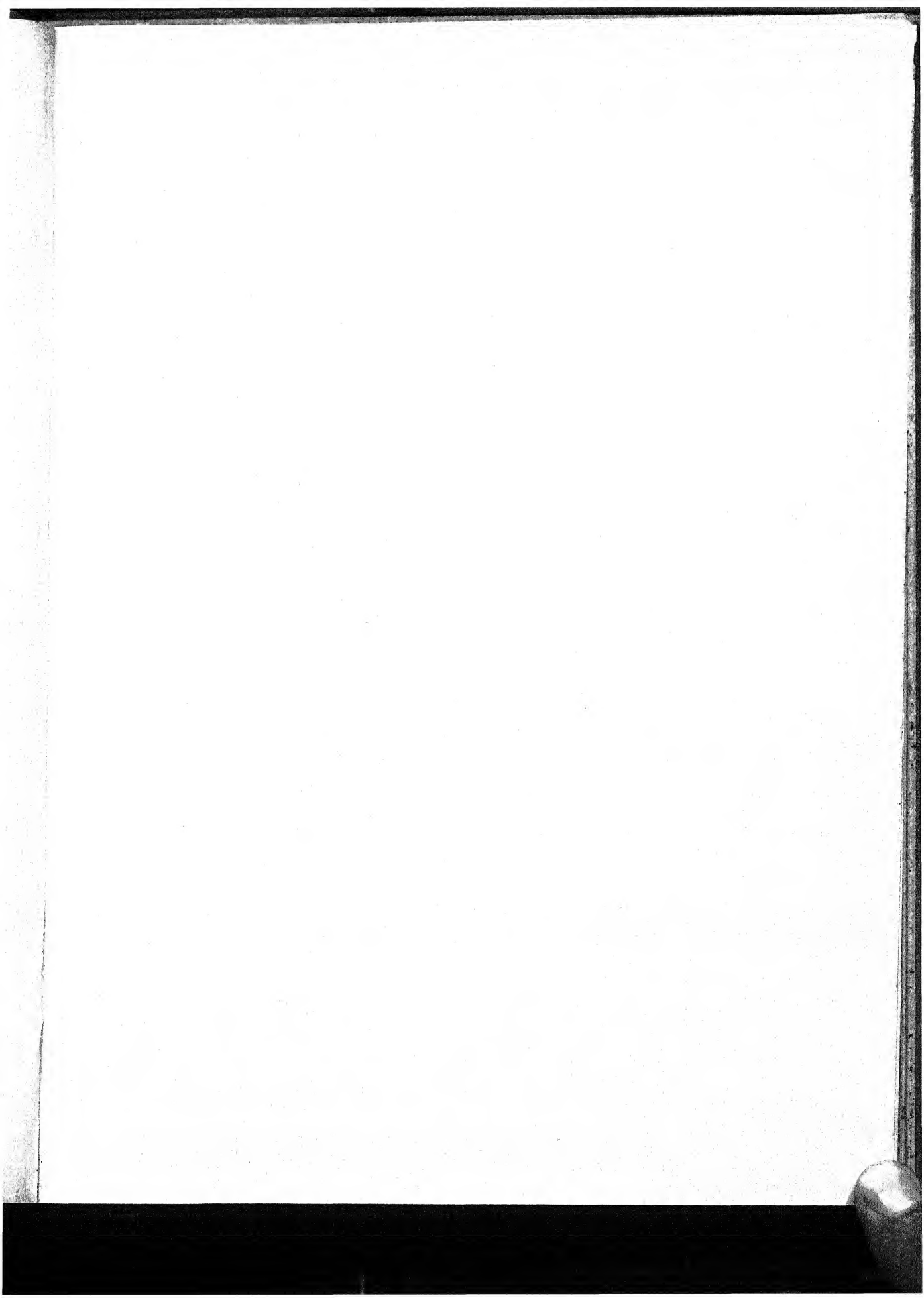
No. of plot	Manurial treatment	Morphine content of opium of 1st lancing	Average of duplicates previous column
		<i>Field No. 29.</i>	
8	No manure . . . . .	7.6	8.6
14	" . . . . .	9.6	
7	Potassium sulphate . . . . .	8.6	9.1
16	" . . . . .	9.7	
6	Superphosphate . . . . .	9.3	9.8
13	" . . . . .	10.3	
5	Nitrate of soda . . . . .	9.1	10.0
15	" . . . . .	11.0	
4	K SO <sub>4</sub> + superphosphate . . . . .	9.7	9.6
10	" . . . . .	9.6	
3	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	9.5	9.6
12	" . . . . .	9.8	
1	Super + NaNO <sub>3</sub> . . . . .	10.0	9.8
11	" . . . . .		
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	10.2	9.6
9	" . . . . .	9.0	

<sup>1</sup> The morphine content rapidly diminishes in the opium from each successive lancing of a given capsule (see *Mem. Dept. Agri. India (Chem. Series)*, vol. VI, no. 1 (pt. III).

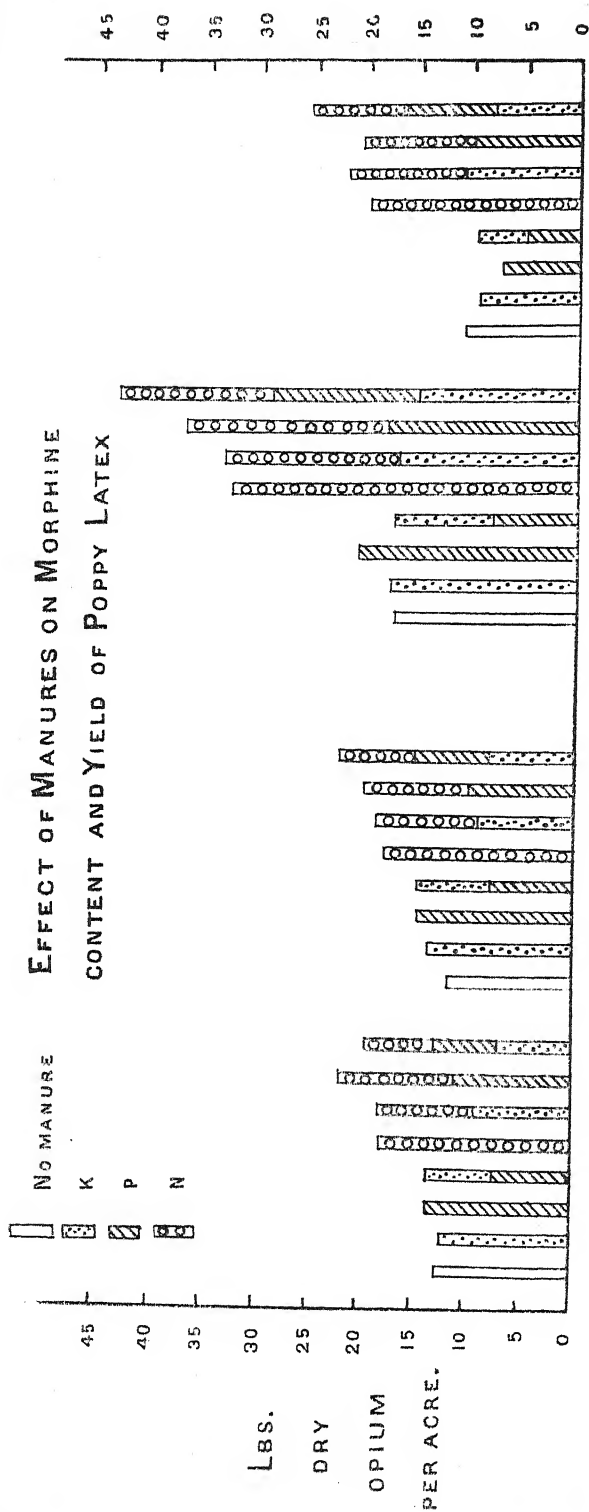
TABLE VIII.—*concd.*

No. of plot	Manurial treatment	Morphine content of opium of 1st lancing	Average of duplicates previous column
<i>Field No. 9.</i>			
8	No manure . . . . .	8.9	9.4
14	„ . . . . .	9.9	
7	Potassium sulphate . . . . .	9.6	9.6
16	„ . . . . .	9.6	
6	Superphosphate . . . . .	11.3	10.8
13	„ . . . . .	10.3	
5	Nitrate of soda . . . . .	10.7	10.8
15	„ . . . . .	11.0	
4	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	9.1	9.7
10	„ . . . . .	10.3	
3	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	10.2	10.8
12	„ . . . . .	11.4	
1	Super + NaNO <sub>3</sub> . . . . .	12.0	11.3
11	„ . . . . .	10.6	
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	11.6	11.2
9	„ . . . . .	10.9	

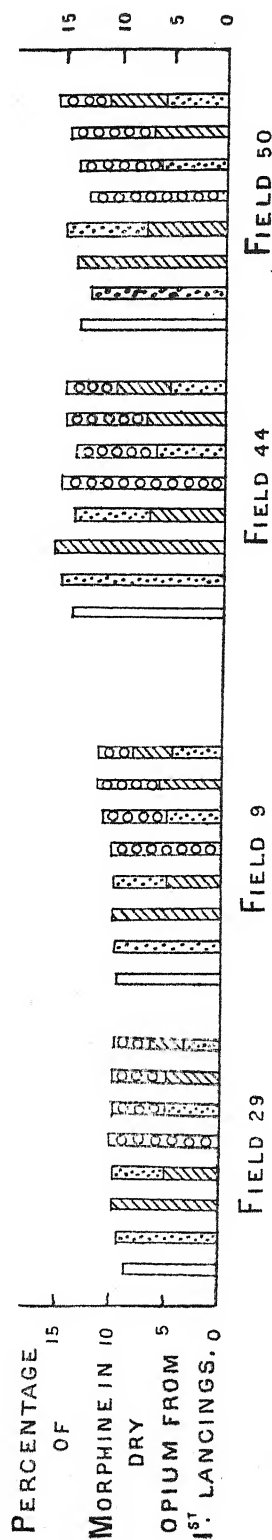
It would seem that, if the morphine content has been affected by any particular system of manuring, the effect is very slight. The effect is not as great as the experimental error (already set out) to be expected in such work. It so happens that, in both fields 9 and 29, the average of the morphine content of the opium from each pair of unmanured plots is the lowest of any of the plots, yet in field 29, unmanured plot No. 14 has given 9.6 per cent. morphine in its opium, which figure is higher than that from plots Nos. 3, 5, and 9 receiving nitrate of soda. Similarly, in field 9 one of the unmanured plots has produced opium of quite a high morphine content. It must be admitted, however, that in both fields there is some slight indication that nitrate of soda has slightly raised the morphine content of the opium. This increase is, however, much within the limits of the experimental error. It is also practically within the amount of the experimental error allowed for the method of analysis employed (*B. P.* 1914), *viz.*, 0.5 per cent. morphine either way.







1917-18.



1916-17

The striking fact however is that nitrate of soda has a truly enormous effect on the yield of opium, yet the effect on the morphine content of that opium is almost inappreciable. This is well emphasized in the diagrams. (Chart V.)

It will be of interest to remark here that the unmanured plots produced undoubtedly ill-nourished plants.

#### 1917-18 EXPERIMENTS.

These experiments were much on the lines of those of the previous year. Two adjoining fields, each of  $\frac{1}{2}$  acre area, were used for the experiment. 10 plots were laid out in each field, measuring  $58' \times 30'$  or almost exactly  $\frac{1}{40}$ th of an acre each. Around each plot was left a margin of crop 2 feet wide which was excluded from the experiment. In this year were also included plots receiving cattle manure and castor cake. Seed of a pure race of poppy was sown over the whole area, but it was not the same as had been used for the manurial experiment in the previous season.

The crop was sown on the 31st October 1917. The land had not been irrigated owing to an opportune fall of rain some few days previously. The seed rate was 6 lb. per acre.

*Manuring.* The diagram shows the arrangement of the plots which was exactly similar in each field.

5	6
4	7
3	8
2	9
1	10

- Plot 1 received cattle manure  
 „ 2 „ superphosphate, potassium sulphate and nitrate of soda.  
 „ 3 „ superphosphate and nitrate of soda.  
 „ 4 „ sulphate of potash and nitrate of soda.  
 „ 5 „ sulphate of potash and superphosphate.  
 „ 6 „ nitrate of soda.  
 „ 7 „ superphosphate.  
 „ 8 „ sulphate of potash.  
 „ 9 was unmanured.  
 „ 10 received castor cake.

*Amounts of manures and time of application.* The dressings this year were even larger than in the previous year. It must be remembered that large dressings were deliberately put on as the main object was to see if manuring could modify the alkaloidal content of the opium produced by the plant.

Superphosphate was	applied at the rate of	8 mds. or 658 lb.	per	acre.
Sulphate of potash	"	"	8 mds. or 658 lb.	" "
Nitrate of soda	"	"	4 mds. or 329 lb.	" "
Castor cake	"	"	20 mds. or 1,646 lb.	" "
Cattle manure	"	"	300 mds. or 11.11 tons	" "

The superphosphate and sulphate of potash were sown on the same day as and just before the seed. The nitrate of soda was put on in two equal dressings, the first on the 3rd January 1918, and the second on the 22nd January 1918. The land was irrigated immediately after each application.

*Observations during growth.* These were practically the same as during the previous season. Potash seemed to have only a slight effect. The effect of superphosphate was distinctly marked in the production of a larger plant. This improvement was noticed when applied alone and with reference to unmanured plots, and also when applied with nitrate of soda and with reference to the plots receiving nitrate of soda alone. The effect of nitrate of soda was again very marked showing up within two to three days of its application. The nitrate of soda plots quickly assumed and retained a dark green foliage and the plants developed very rapidly. Plots No. 10 receiving castor cake were very similar to the plots No. 2 receiving the nitrate, superphosphate, and potassium sulphate.

No connection could be observed between time of flowering and application of any particular manure.

*Harvesting of opium.* The fields were not all ready for harvesting at the same time. Field 44 was harvested in the usual Indian way.<sup>1</sup> The knife used had 4 parallel blades.

In field 50 the capsules at each lancing received three parallel vertical cuts with the knife, with the idea of increasing the yield of opium.

On both fields the number of capsules lanced at the first lancing in each plot was recorded, so that data could be obtained of the yield of opium per

<sup>1</sup> Annett, Sen and Singh. *Mem, Dept. Agri. India, Chem. Ser., vol. VI, no. 1* (part II).

capsule at the first lancing. This could not be done at every lancing, owing to the mass of work involved in such a proceeding.

The opium was allowed to dry in the laboratory at the ordinary temperature of the room, *viz.*, about 100 °F., by being spread out in a thin layer. When dry enough, it was torn into small pieces and these were again spread out to dry until the whole sample could be powdered and sieved. The samples then contained from 92 to 95 per cent. dry matter. The total dry matter and morphine content of each sample were then estimated. For the morphine estimation the air-dry powdered samples were taken. The loss of weight on drying the sample in the oven was estimated in a separate 2 gm. portion.

The codeine and narcotine content of the first lancements on field 44 were also determined.

*Methods of analysis.*

*Morphine* was estimated by the method of the *British Pharmacopœia*, 1914.

*Codeine* was estimated by a new method we have devised.<sup>1</sup>

*Narcotine* was estimated in the ether extract of the dried opium by extracting the ether extract with acid, addition of ammonia to the acid extract and re-extracting the narcotine with ether.

*Straw, capsules, and seed.* After harvesting the opium, the crop was left until dead ripe as in the previous year. The hot sun had by then dried up all green parts of the plant. The total air-dry weight of the above ground portions of the plants was then recorded, and also the total air-dry weight of the capsules. The number of plants and of capsules in each plot was also recorded in order to determine if the number of capsules borne per plant had any connection with the manure used. Finally the seed was separated from the capsules and the weight of seed on each sub-plot was determined.

The attached tables give the results of the experiments. Chart V expresses the results diagrammatically.

<sup>1</sup>The *Analyst*, XLV, 1920, p. 321.

TABLE IX.

*Effect of manure on morphine, codeine and narcotine content of opium.*

No. of plot	Manurial treatment	PER CENT. OF MORPHINE IN OPIUM DRIED AT 100°C. OF EACH SUC- CESSIVE LANCING						Per- centage of codeine in opium of 1st lancing	Per- centage of nar- cotine in opium of 1st lancing	Total mor- phine narco- tine and codeine in 1st lancing
		1st	2nd	3rd	4th	5th	6th			
Field 44. Country lancing.										
9	Unmanured . . .	14.1	12.4	9.4	6.3	3.0	2.3	2.14	4.63	18.8
8	Sulphate of potash . .	14.9	11.5	8.6	7.3	4.5	1.4	2.20	4.36	21.5
7	Superphosphate . . .	15.8	12.0	8.0	6.3	4.2	1.2	1.48	5.00	22.3
6	Nitrate of soda . . .	15.0	11.11	8.7	6.4	4.0	..	2.18	5.06	22.2
5	K <sub>2</sub> SO <sub>4</sub> + super . . .	13.8	12.1	8.7	5.9	3.6	..	1.72	3.34	18.7
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	13.8	11.4	7.9	6.3	4.8	2.1	1.81	7.74	22.8
3	Superphosphate + nitrate of soda . . .	14.9	13.0	8.1	6.1	3.3	2.5	2.21	5.70	22.9
2	Superphosphate + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	14.7	11.7	9.5	5.4	2.3	1.6	2.19	4.92	21.8
1	Cattle dung . . .	14.7	11.8	8.8	6.2	3.7	2.0	2.91	4.79	22.4
10	Castor cake . . .	14.5	12.5	10.3	5.1	4.1	2.6	2.33	3.57	20.4
Field 50. Treble lancing.										
9	Unmanured . . .	13.4	11.2	9.1	..	..	..	Not estimated		
8	Sulphate of potash . .	12.6	9.4	5.6	..	..	..	..	..	..
7	Superphosphate . . .	13.9	10.3	9.0	..	..	..	..	..	..
6	Nitrate of soda . . .	12.5	10.6	10.2	..	..	..	..	..	..
5	K <sub>2</sub> SO <sub>4</sub> + super . . .	14.8	12.1	8.3	..	..	..	..	..	..
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	13.7	11.3	9.4	..	..	..	..	..	..
3	Super + NaNO <sub>3</sub> . . .	14.7	10.4	11.1	..	..	..	..	..	..
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	15.7	13.4	9.8	8.4	6.2	..	..	..	..
1	Cattle dung . . .	14.7	13.3	9.9	7.8	8.4	..	..	..	..
10	Castor cake . . .	16.7	10.9	9.5	6.6	8.0	..	..	..	..

*Note.* The marginal areas around the plots were also lanced for opium. The plants there had, of course, received no manure and being close up to the edges of the field were mostly small and stunted largely owing to edges of the field being badly cultivated. Two samples of opium, 1st lancements only, collected from around and between the plots of fields 44 and 50, were found to contain 14.5 and 13.2 per cent. morphine respectively.

TABLE X.

*Effect of manures on out-turn of opium.*

No. of plot	Manurial treatment	WEIGHT OF OPIUM IN GRM. AT EACH SUCCESSIVE LANCING DRIED AT 100°C.							Total weight of dry opium per plot grm.	Lb. of dried opium per acre	Yield of dry opium unmanured plot = 100
		1st	2nd	3rd	4th	5th	6th	7th			
Field No. 44. Country lancing.											
9	Unmanured . .	67.7	50.4	32.7	24.6	12.0	6.4	..	193.8	17.11	100
8	K <sub>2</sub> SO <sub>4</sub> . . .	72.1	67.8	35.7	12.5	9.0	2.2	0.5	199.8	17.64	103
7	Superphosphate .	70.7	83.1	52.7	10.7	11.6	3.6	0.7	233.1	20.59	120
6	NaNO <sub>3</sub> . . .	169.7	34.9	39.1	12.4	6.8	1.7	0.4	365.0	32.22	188
5	K <sub>2</sub> SO <sub>4</sub> + super .	78.5	66.2	24.1	13.8	7.4	1.6	0.6	192.2	16.97	99
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> .	132.9	126.3	68.0	29.7	11.8	2.7	0.4	371.8	32.83	192
3	Super + NaNO <sub>3</sub> .	164.5	124.1	89.5	33.95	0.2	3.2	..	420.4	37.11	217
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	180.4	157.0	53.9	54.43	0.31	3.4	..	489.4	43.22	253
1	Cattle dung . .	91.4	85.1	24.7	18.81	0.5	4.0	..	234.5	20.71	121
10	Castor cake . .	123.7	88.9	40.3	22.2	9.8	3.2	..	288.1	25.4	149
Field No. 50. Treble lancing.											
9	Unmanured . .	79.6	34.7	5.1	..	..	..	..	119.4	10.54	100
8	K <sub>2</sub> SO <sub>4</sub> . . .	88.0	17.6	3.5	..	..	..	..	109.1	9.63	91
7	Superphosphate .	53.4	20.3	6.0	..	..	..	..	79.7	7.04	67
6	NaNO <sub>3</sub> . . .	182.2	34.7	8.8	..	..	..	..	225.7	19.93	189
5	K <sub>2</sub> SO <sub>4</sub> + super .	75.1	29.4	5.3	..	..	..	..	109.8	9.70	92
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> .	188.9	45.2	13.3	..	..	..	..	247.4	21.85	207
3	Super + NaNO <sub>3</sub> .	146.4	74.8	9.1	..	..	..	..	230.3	20.33	193
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	122.7	91.5	68.4	2.5	2.2	..	..	287.3	25.36	241
1	Cattle dung . .	93.9	37.0	32.5	2.2	9.1	..	..	174.7	15.43	146
10	Castor cake . .	198.5	49.1	44.3	2.2	4.2	..	..	298.3	26.60	250



TABLE XI.

*Shewing effect of manures on out-turn of opium per 1,000 capsules.*

No. of plot	Manurial treatment	OPIUM OF 1ST LANCING				TOTAL YIELD			
		No. of capsules per plot lanced at 1st lancing	Yield of dry opium grm.	Yield of dry opium per 1,000 capsules grm.	Yield of unmanured plot = 100	No. of capsules per plot	Yield of dry opium grm.	Yield of dry opium per 1,000 capsules grm.	Yield of unmanured plot = 100
Field 44. Country lancing.									
9	Unmanured . . . .	1,910	67.7	35.4	100	5,755	193.8	33.7	100
8	K <sub>2</sub> SO <sub>4</sub> . . . .	2,698	72.1	26.7	75	4,645	199.9	43.0	128
7	Superphosphate . . . .	2,241	70.7	31.5	89	5,245	233.1	44.4	132
6	NaNO <sub>3</sub> . . . .	4,044	169.7	42.0	118	5,903	365.0	61.9	184
5	K <sub>2</sub> SO <sub>4</sub> + super . . . .	3,180	78.5	24.7	70	5,348	192.3	36.0	107
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	3,276	132.0	40.6	114	6,470	371.8	57.5	171
3	Super + NaNO <sub>3</sub> . . . .	3,530	164.5	46.6	131	5,909	420.4	71.1	211
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	3,240	180.4	55.7	157	5,214	489.4	93.9	279
1	Cattle dung . . . .	2,736	91.4	33.4	94	5,232	234.6	44.8	133
10	Castor cake . . . .	3,212	123.7	38.5	109	5,752	288.2	70.0	208
Field 50. Treble lancing.									
9	Unmanured . . . .	2,732	79.6	29.1	100	6,134	119.4	19.5	100
8	K <sub>2</sub> SO <sub>4</sub> . . . .	3,812	88.0	23.1	79	6,770	107.1	16.1	83
7	Superphosphate . . . .	2,910	53.4	18.3	63	7,285	79.7	10.9	56
6	NaNO <sub>3</sub> . . . .	4,090	182.2	44.3	153	7,673	225.7	29.4	151
5	K <sub>2</sub> SO <sub>4</sub> + Super . . . .	2,545	75.1	29.5	101	8,864	109.8	12.4	64
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	4,044	188.9	46.7	160	5,967	247.4	41.5	213
3	Super + NaNO <sub>3</sub> . . . .	3,220	146.4	45.4	156	6,813	230.3	33.8	174
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	2,343	122.7	52.4	160	7,025	287.3	40.9	210
1	Cattle dung . . . .	1,941	93.9	48.4	166	5,570	174.7	31.3	161
10	Castor cake . . . .	3,470	198.5	57.2	198	6,225	298.3	47.9	246

One great difference will be observed between fields 44 and 50 in the above tables. The figures in column 6 differ very considerably from those in column 10, in the case of field 44, whereas in field 50 they fairly well correspond in most cases. This is because in No. 50 the main bulk of the yield of opium was obtained at the first lancing. It will be remembered that the capsules in this plot all received three incisions at each lancing, and in this method the yield of opium rapidly falls off at each subsequent lancing. In field 44, however, the ordinary country lancing method of making one incision with a 4-bladed knife was used, and it is our experience that the individual capsules frequently give more opium by this method at the second lancing than at the first. For this reason the yield of opium of the individual capsule at the first lancing is not a fair indication of the total yield of opium to be expected from these same capsules on each manurial plot.

TABLE XII.

*Shewing relation between yield of opium per 1,000 capsules and per plot.*

No. of plot	Manurial treatment	Total weight of opium dried at 100°C. per plot gm.	Yield of opium per plot unmanured = 100	Yield of opium per 1,000 capsules gm.	Yield of opium per 1,000 capsules unmanured = 100
<i>Field No. 44.</i>					
9	Unmanured . . . . .	193.8	100	33.7	106
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	199.8	103	43.0	128
7	Superphosphate . . . . .	233.1	120	44.4	132
6	NaNO <sub>3</sub> . . . . .	365.0	185	61.9	184
5	Super + K <sub>2</sub> SO <sub>4</sub> . . . . .	192.3	99	36.0	107
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	371.8	192	57.5	171
3	Super + NaNO <sub>3</sub> . . . . .	420.4	217	71.1	211
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	489.4	253	93.9	279
1	Cattle dung . . . . .	234.6	121	44.8	133
10	Castor cake . . . . .	288.2	149	70.0	208

<i>Field No. 50.</i>					
9	Unmanured . . . . .	119.4	100	19.5	100
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	109.1	91	16.1	83
7	Superphosphate . . . . .	79.7	67	10.9	56
6	NaNO <sub>3</sub> . . . . .	285.7	189	29.4	151
5	Super + K <sub>2</sub> SO . . . . .	109.8	92	12.4	64
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	247.4	207	41.5	213
3	Super + NaNO <sub>3</sub> . . . . .	230.3	193	33.8	174
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	287.3	241	40.9	210
1	Cattle dung . . . . .	174.7	146	31.3	161
10	Castor cake . . . . .	298.3	250	47.9	246

TABLE XIII.

*Shewing effect of manure on weights of plants and capsules per plot.*

Plot No.	Manurial treatment	Weight of plants with capsules per plot lb.	Yield of opium per plot. unmanured plot = 100	Weight of capsules per plot lb.	Wt. of capsules per plot unmanured = 100
<i>Field No. 44.</i>					
9	Unmanured . . . . .	55.6	100	29.9	100
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	63.9	115	30.9	103
7	Superphosphate . . . . .	74.2	133	36.0	120
6	NaNO <sub>3</sub> . . . . .	96.8	174	49.2	164
5	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	68.0	122	37.1	123
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	92.7	167	45.8	153
3	Super + NaNO <sub>3</sub> . . . . .	118.2	213	56.6	186
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	121.5	218	54.1	180
1	Cattle dung . . . . .	74.2	133	36.0	120
10	Castor cake . . . . .	93.7	168	42.2	140
<i>Field No. 50.</i>					
9	Unmanured . . . . .	59.7	100	31.4	100
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	62.3	104	32.9	105
7	Superphosphate . . . . .	63.9	107	30.9	98
6	NaNO <sub>3</sub> . . . . .	96.8	162	48.4	154
5	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	64.9	109	30.9	98
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	99.4	166	49.4	157
3	Super + NaNO <sub>3</sub> . . . . .	121.0	203	55.1	175
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	117.4	197	52.5	167
1	Cattle dung . . . . .	64.9	109	34.0	108
10	Castor cake . . . . .	112.8	189	53.6	170

TABLE XIV.

*Effect of manures on number of plants and capsules per plot.*

No. of plot	Manurial treatment	Number of plants	No. of plants per plot un-manured = 100	No. of capsules	No. of capsules per plot un-manured = 100	No. of capsules per 100 plants
<i>Field No. 44.</i>						
9	Unmanured . . . . .	5,502	100	5,755	100	105
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	4,413	80	4,645	81	105
7	Superphosphate . . . . .	4,700	85	5,245	91	112
6	NaNO <sub>3</sub> . . . . .	5,716	104	5,903	103	103
5	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	4,994	91	5,348	93	107
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	6,279	114	6,470	112	103
3	Super + NaNO <sub>3</sub> . . . . .	5,517	100	5,909	103	107
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	*	..	5,214	91	..
1	Cattle dung . . . . .	5,142	93	5,232	91	102
10	Castor cake . . . . .	5,361	97	5,752	100	107

<i>Field No. 50.</i>						
9	Unmanured . . . . .	6,035	100	6,134	100	102
8	K <sub>2</sub> SO <sub>4</sub> . . . . .	6,571	109	6,770	110	103
7	Superphosphate . . . . .	7,192	119	7,285	119	101
6	NaNO <sub>3</sub> . . . . .	7,571	125	7,673	125	101
5	K <sub>2</sub> SO <sub>4</sub> + super . . . . .	7,928	131	8,864	144	112
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	5,830	97	5,967	99	102
3	Super + NaNO <sub>3</sub> . . . . .	6,506	103	6,613	111	105
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . . .	6,617	110	7,025	114	106
1	Cattle dung . . . . .	5,146	85	5,570	91	108
10	Castor cake . . . . .	5,778	96	6,225	101	108

\* Not available.

TABLE XV.

*Relation between yield of opium, capsules and seed per acre.*

Plot No.	Manurial treatment	OUT-TURN OF OPIUM DRIED AT 100°C.		OUT-TURN OF CAPSULES		OUT-TURN OF SEED.	
		lb. per acre	Un-manured plot = 100	lb. per acre	Un-manured plot = 100	lb. per acre	Un-manured plot = 100
Field No. 44. Country lancing.							
9	Unmanured . . . .	17.11	100	1,196	100	555.6	100
8	K <sub>2</sub> SO <sub>4</sub> . . . .	17.64	103	1,236	103	632.8	114
7	Superphosphate . . . .	20.59	120	1,440	120	689.6	124
6	NaNO <sub>3</sub> . . . .	32.23	188	1,968	164	982.8	177
5	K <sub>2</sub> SO <sub>4</sub> + super . . . .	16.97	99	1,484	123	957.2	172
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	32.83	192	1,832	153	895.6	161
3	Super + NaNO <sub>3</sub> . . . .	37.11	217	2,264	186	1126.4	203
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . . .	43.25	253	2,164	180	1069.6	203
1	Cattle dung . . . .	20.71	121	1,440	120	704.4	127
10	Castor cake . . . .	25.45	149	1,688	140	843.6	152

*Field No. 50. Triple lancing.*

9	Unmanured . . .	10.54	100	1,256	100	617.2	100
8	K <sub>2</sub> SO <sub>4</sub> . . .	9.63	91	1,316	105	637.6	103
7	Superphosphate . . .	7.04	67	1,236	98	580.8	94
6	NaNO <sub>3</sub> . . .	19.93	189	1,936	154	869.2	141
5	K <sub>2</sub> SO <sub>4</sub> + super . . .	9.70	92	1,236	98	586.8	95
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	21.85	207	1,976	157	957.2	155
3	Super + NaNO <sub>3</sub> . . .	20.33	193	2,204	175	1131.6	183
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	25.36	241	2,100	167	1049.2	170
1	Cattle dung . . .	15.43	146	1,360	108	637.6	103
10	Castor cake . . .	26.60	250	2,144	170	1039.2	169

## CONCLUSIONS DRAWN FROM 1917-18 EXPERIMENTS.

*A. Effect of manures on out-turn of opium.*

Tables X and XI deal with this effect and Chart V sets out the results diagrammatically. The previous year's conclusions were amply confirmed by the experiments of 1917-18. Nitrate of soda has again had a very marked effect in increasing the out-turn of opium in every case in which it was applied. The amount used seems to have almost doubled the yield of opium.

Sulphate of potash again has had no effect when applied alone, and practically none when applied with nitrate of soda. When applied with superphosphate and nitrate of soda together it seems, however, to have had an effect, for the yields on plot 2 in each field are greater than on plot 4. Plot 2 received all three artificial manures, while plot 4 received superphosphate and nitrate of soda, but no potash.

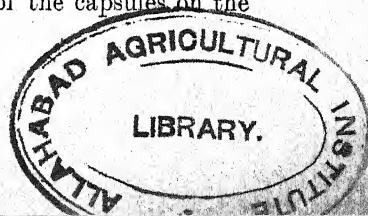
Superphosphate, applied alone, appears to have had a slight beneficial effect on field 44 but none on field 50. Applied in conjunction with potash, it seems to have had no effect on either field. But when applied together with potash and nitrate of soda (plot 2 on each field) it has given a distinct increase in yield on both fields over the plots receiving potash and nitrate of soda.

The experiments again show that the increased out-turn, produced by nitrate of soda, is due to an increase of yield of the individual capsules. Thus Table XIV shows the number of capsules on each of the differently manured plots. It will be seen that the differences are not great. Moreover, Table XII shows that a very close relation exists between the yield of opium per plot and per 1,000 capsules in each plot.

Table XV shows also that there is again a fairly general relation between the yield of opium per plot and the total weight of capsules in each plot. Since manuring does not seem to affect the number of capsules per plot (*see* Table XIV), the yield of opium for a given pure race of poppy would appear to be proportional to the size and weight of the capsule.

*B. Effect of manures on out-turn of seed.*

This is shown in Table XV. Manures seem to have had the same effect on the seed out-turn as they had on the out-turn of opium. There is but one exception, that on plot 5 of field 44 receiving potash and superphosphate. This plot has given a very high yield of seed. With this exception Table XV shows that the yield of seed is almost exactly proportional to the yield of opium and fairly well proportional to the total weight of the capsules on the





various plots. The relation is not so good in field 50 as in field 44, probably owing to the fact that the method of lancing used on field 50 caused capsules to yield less opium than they would have yielded by the ordinary method of lancing.

*C. Effect of manures on the alkaloid content of the opium.*

1. *Morphine.* The results are an ample confirmation of our conclusions from last year's result and are well illustrated diagrammatically in Chart V. That is, the percentage of morphine in the opium has not been modified to an appreciable extent by any system of manuring. This is well seen in Table IX. On referring to page 99, it will be seen that in a control experiment it was found that the morphine content of opium produced in eight different plots of the same field uniformly treated varied from 11.5 to 14.3.

A control experiment was also carried out in the season 1918-19.

The opium collected from the first lancements of the eight plots into which the control field was divided gave the following percentage of morphine:—12.8, 12.1, 12.9, 12.2, 11.8, 12.0, 12.6, and 12.6.

The results were therefore much more uniform than those of the previous year's control experiment. The range of variation in morphine percentage of the opium produced at the first lancing on the various manurial plots of field 44 was very small, *viz.*, 14.1 to 15.8. On field 50, due no doubt to the different method of lancing adopted, the range of variation was greater, *viz.*, 12.6 to 16.7.

There is, moreover, not much variation in the various plots of the morphine content of the opium of the second, third, and subsequent lancements. One big difference will be observed in the table between fields 50 and 44. The morphine content of the opium from the fourth and fifth lancements of the former field are considerably higher than those on plot 44. This is explained by the fact that on field 50 treble lancing was employed as previously stated. This method of lancing soon dries up the capsules, say after the second or at latest third lancing, and many capsules yield no further latex by this system even at the second lancing. Therefore in field 50, at the fourth and subsequent lancements, as compared with field 44, we shall have a higher proportion of heads being lanced for the first time. It has been shown that heads lanced for the first time produce opium with the highest percentage of morphine, and hence in the fourth and fifth lancements from field 50 one would expect the opium to contain more morphine than the opium from the fourth and fifth lancements of field 44.

Finally, nitrate of soda has again had an enormous effect on the out-turn of opium, but, at the same time, the percentage of morphine in that opium seems to have undergone no change at all. This is well illustrated in Chart V.

2. *Codeine*. The codeine content of the opium from the various plots has varied from 1.48 to 2.91. This latter figure was obtained on the plot receiving cattle dung. Omitting this figure and the figure from the castor cake plot the range of variation on the remaining plots was from 1.48 to 2.29. The error in the method of analysis is probably not more than 0.10 per cent. The codeine content on plots 2, 3, 6, 8 and 9 are practically identical, and there seems to be no connection between any particular manurial application and the codeine content of the opium produced.

3. *Narcotine*. The narcotine content has varied from 2.55 to 7.16 per cent. The unmanured plot has produced opium of the lowest narcotine content. The variations in the narcotine content do not however appear to have any relation to any particular manurial application.

#### 1919-20 EXPERIMENTS.

In the seasons 1918-19 and 1919-20 the experiment was limited to determining the effect of nitrogen in increasing amounts applied in the form of  $\text{NaNO}_3$  and also in the form of poppy cake and castor cake. The 1918-19 results were unsatisfactory since the crop was very uneven, but in any case the results are not available owing to damage caused to the samples by a fire which broke out in the laboratory.

In the season 1919-20 the field used was half an acre in area and was divided into 16 equal plots: the whole  $\frac{1}{2}$  acre was sown with seed of the same pure race of poppy and a margin of non-experimental plot was left all round each plot. Opium was however collected from the capsules in these marginal areas and analyses of the samples so collected provided a check on the results from the manurial plots and also acted as a control on the possibility of creepage effects of manures.

The amounts of manure applied are shown in the tables. The nitrate of soda was in each case put on in two dressings, the first on 21-12-19 and the second on 13-1-20.

#### *Harvesting of produce.*

*Opium*. When lancing commenced, as many terminal capsules in each plot as were ready were selected and labelled and counted. These heads received four successive lancements. In this way we were able to determine the yield per 1,000 heads at each successive lancing in each plot, and also we were certain that the produce of the second lancing did not contain opium from heads lanced for the first time and so on. Later on more terminal heads came to maturity and on some of the plots these were lanced. No subsequent lancing was carried out in these however. Thus a duplicate set of data was

obtained for some of the plots. The remaining capsules on the plot were not lanced since we were concerned with the yield of latex per plant rather than with the total produce per plot.

*Straw, capsules, and seed.* After harvesting the opium the plants were allowed to dry in the hot sun on the plots and were then cut and removed to the threshing yard. Here they were allowed to dry further in the sun and the total produce of each plot was weighed. The total weight of capsules and of seed per plot were also determined and also from each plot the weight of 1,000 terminal capsules and of the resulting seed.

The total number of plants and of capsules per plot were also counted in order to see if the varying amount of manure affected the number of capsules per plot. As in 1916-17 this was found not to be the case and therefore the figures will not be reproduced here. The following tables set out our results.

TABLE XVI.

*Effect of nitrogenous manures on morphine and codeine content of opium.*

Treatment per acre	PERCENTAGE OF MORPHINE IN DRY MATTER OF OPIUM				Second crop of capsules first lancing	Peren- tage of codeine in dry matter of opium of first lancing
	No. of lancing					
	1	2	3	4		
Unmanured . . . . .	14.4	9.4	4.3	3.6	14.4	3.70
80 lb. "NaNO <sub>3</sub> . . . . .	14.4	10.9	6.5	4.5	..	3.46
80 lb. "NaNO <sub>3</sub> . . . . .	15.9	11.3	6.5	4.4	..	3.89
160 lb. "NaNO <sub>3</sub> . . . . .	14.6	10.2	6.7	4.7	14.6	3.24
160 lb. "NaNO <sub>3</sub> . . . . .	15.9	10.5	6.4	3.8	..	3.96
320 lb. "NaNO <sub>3</sub> . . . . .	15.0	11.0	6.8	4.4	..	3.77
320 lb. "NaNO <sub>3</sub> . . . . .	15.0	9.9	4.8	3.8	15.0	3.50
480 lb. "NaNO <sub>3</sub> . . . . .	15.9	12.1	7.1	5.0	14.9	3.41
480 lb. "NaNO <sub>3</sub> . . . . .	15.8	11.2	6.4	3.8	14.8	3.74
640 lb. "NaNO <sub>3</sub> . . . . .	16.1	10.3	6.8	4.4	..	3.26
640 lb. "NaNO <sub>3</sub> . . . . .	16.6	11.1	5.8	3.2	15.3	3.84
1,600 lb. poppy cake supplying N = 530 lb. NaNO <sub>3</sub> . . . . .	16.4	10.1	5.7	3.8	..	3.48
1,600 lb. poppy cake supplying N = 530 lb. NaNO <sub>3</sub> . . . . .	16.7	11.6	6.0	5.3	15.6	3.52
1,600 lb. castor cake supplying N = 580 lb. NaNO <sub>3</sub> . . . . .	15.7	10.9	6.7	4.1	..	3.81
1,600 lb. castor cake supplying N = 580 lb. NaNO <sub>3</sub> . . . . .	17.5	11.9	7.5	4.2	..	3.95
" . . . . .	17.1	11.9	7.3	5.0	..	4.27

*Note.* On each plot the opium was collected in duplicate samples which were analysed separately. The average of these two samples only is given, since they agreed very closely in practically every case.

In addition, eight samples of opium of the first lancings only, harvested from the margins between the plots, gave the following figures for morphine content, 14.8, 16.0, 14.4, 14.9, 14.8, 15.0, 15.3 and 14.8, with an average of 15.0, which fairly well agrees with the average figures obtained for the unmanured plots. Six of these samples from the marginal areas were also examined for codeine content and gave the following figures, 3.57, 3.51, 3.83, 4.20, 4.01 and 4.70.

TABLE XVII.

*Effect of manures on yield of opium per 1,000 capsules.*

Treatment per acre	GM. OPIUM RECKONED AS DRY MATTER PER 1,000 CAPSULES					UNMANURED PLOT = 100	
	No. of lancing					1st lancing yield	Total yield of all lancings
	1	2	3	4	Total		
Unmanured . . . .	35.2	26.4	7.6	2.2	71.4	100	100
80 lb. $\text{NaNO}_3$ . . .	37.3	28.5	15.3	9.2	90.3	106	127
160     ,,     . . .	43.6	34.7	14.4	8.5	101.2	124	142
320     ,,     . . .	39.9	21.7	9.0	2.9	73.5	113	103
480     ,,     . . .	50.6	32.0	8.1	2.4	93.1	144	130
640     ,,     . . .	55.7	34.1	6.0	5.3	101.1	158	142
1,600 lb. poppy cake con- taining N = 530 lb. $\text{NaNO}_3$ . . . .	53.8	44.5	13.3	6.9	118.5	153	166
1,600 lb. castor cake con- taining N = 580 lb. $\text{NaNO}_3$ . . . .	51.6	49.5	20.3	16.4	137.8	146	193

*Note.* These figures are only approximate. Unfortunately portions of some of the samples were stolen from the laboratory and before the actual moisture content of the samples had been determined. Luckily, however, the field weights of the samples in the moist condition were available and we are convinced that all the weights are within 5 per cent. of the truth.

TABLE XVIII.

*Relation between weights of plants, capsules, seed and opium. Unmanured plot 100.*

Treatment per acre	Total plant produce per plot	Weight of 1,000 complete plants (sun-dried)	Weight of 1,000 terminal capsules (sun-dried)	Weight of seed from 1,000 terminal capsules.	YIELD OF OPIUM (DRY MATTER) PER 1,000 CAPSULES	
					At 1st lancing	Total of all lancements
Unmanured . . . . .	100	100	100	100	100	100
80 lb. $\text{NaNO}_3$ . . . . .	115	142	121	124	106	127
160     "     . . . . .	122	154	134	143	124	142
320     "     . . . . .	137	124	106	122	113	103
480     "     . . . . .	133	143	120	138	144	130
640     "     . . . . .	133	141	118	132	158	142
1,600 lb. poppy cake supplying N = 530 lb. $\text{NaNO}_3$ . . . . .	150	200	161	169	153	166
1,600 lb. castor cake supplying N = 580 lb. $\text{NaNO}_3$ . . . . .	151	230	181	177	146	193

## DISCUSSION OF 1919-20 RESULTS.

Table XVI certainly indicates that with increasing amounts of nitrogenous manure there is a slight tendency for the morphine content of the latex of the first lancing to increase. Castor cake, although it did not supply as much total nitrogen as the maximum amount of  $\text{NaNO}_3$ , yet seems to have given opium of the highest morphine concentration. The difference in morphine content observed in the opium of the various plots though small is yet distinctly significant since in each plot the opium was collected in two samples, and each sample was analysed separately and in practically every case the analyses of the duplicate samples were in close agreement. Moreover the analyses of 8 samples of opium obtained from marginal areas surrounding the plots gave an average figure of 15.0 per cent. morphine which is not much higher than the average of the figures obtained in the unmanured plots, *viz.*, 14.4.

It is difficult to trace any connection between the manures applied and the codeine content of the opium, but the castor cake plots have produced opium of distinctly higher codeine content than the other plots.



Turning to the yield of opium, however, as in previous years one notices very marked differences in the different plots.

The results on the various nitrate of soda plots are rather variable. The maximum total yield of opium per 1,000 capsules was given on the plots receiving 160 lb.  $\text{NaNO}_3$ . Increase of nitrate beyond this amount seems to have indefinite results though certainly 640 lb.  $\text{NaNO}_3$  per acre gave a higher yield of opium at the first lancing than any of the other plots, the cake plots included. The main point brought out, however, is that again, as in previous seasons, nitrogenous manures largely increase the yield of latex but the morphine concentration of the latex is only slightly modified.

Table XVIII shows the relation between the weight of the individual capsules, their seed yield and their opium yield, the produce of the unmanured plot being taken as 100 in each case. There again seems to be a close connection between these three sets of data.

#### SUMMARY.

1. The morphine content of opium is not altered to any marked extent by manuring with sulphate of potash, superphosphate, or nitrate of soda singly, or in combination, or by cattle manure, poppy cake or castor cake. Large dressings of nitrogen tend to slightly increase the morphine content of the latex but any variations observed are practically within the limits of the experimental error to be expected in this kind of work. Control experiments have been made in order to determine the magnitude of the experimental error.

As regards codeine and narcotine content of opium, the data are not sufficient to enable definite conclusions to be drawn. There were variations on the differently manured plots, but these did not seem to be connected with any particular form of manuring.

2. The yield of opium can be very greatly increased by nitrogenous manuring. Superphosphate seems to have a slight effect in the same direction, but potash seems to have exercised no effect whatever. Nitrogen is undoubtedly the dominant element for the poppy plant. At the same time, continuous heavy manuring with nitrate of soda would, with the consequent production of larger crops, probably exhaust the amount of other plant foods, *e.g.*, phosphate or potash, available for the plant in the soil. Hence it would probably prove better in the long run to apply the nitrogenous manure in some form of cake, *e.g.*, as castor cake. It will be noted that the yield of opium per plot or per capsule may be doubled by the use of nitrate of soda, and yet the opium produced shows but little variation in its morphine content. This is well



illustrated below by the nitrate of soda plots Nos. 9, 6, 4, 3, and 2 of field 44, and also by the diagram in Chart V.

No. of plot	Manurial treatment	Yield of dry opium per plot unmanured plot = 100	Yield of dry opium per 1,000 capsules unmanured plot = 100	Percentage of morphine in dry opium
9	Unmanured . . . . .	100	100	14.7
6	Nitrate of soda . . . . .	188	184	15.3
4	$K_2SO_4 + NaNO_3$ . . . . .	192	171	14.3
3	Super + $NaNO_3$ . . . . .	217	211	15.9
2	Super + $K_2SO_4 + NaNO_3$ . . . . .	253	279	15.7

3. For the same pure race of poppy the yield of opium is more or less proportional to the size of capsule. The various manures have little effect on the total number of capsules per plot or per plant. Poor ill-nourished capsules yield less opium than large robust ones. Yet the morphine content of the opium produced is practically the same in both cases.

4. The yield of seed per plot, or per capsule, has been shown to be more or less proportionate to the yield of opium. This perhaps is only to be expected since the yield of opium bears such a close relation to the size of the capsules. It is, however, a point of interest in relation to Muller's theory<sup>1</sup> that the alkaloids of the latex are the source of the nitrogenous compounds of the seed.

#### THE INFLUENCE OF STARVATION.

The plants on the unmanured plots in all our manurial experiments were certainly very poor specimens and ill-nourished. It seemed of interest to deliberately produce the poorest plants possible and to examine the latex produced by them. For this purpose some hard uncultivated land at Cawnpore was ploughed up, and poppy grown on it in the season 1918-19. A most miserable crop resulted and we were able to obtain complete plants only some 5" to 8" high. A portion of this field was dressed with nitrate of soda and this certainly improved the plants growing there but only slightly. The capsules lanced were mostly from  $\frac{1}{3}$ " to  $\frac{2}{3}$ " long, though they were slightly larger on the plants receiving nitrate of soda.

The opium of the first lancing from the unmanured portion yielded 8.5 per cent. morphine. The portion receiving nitrate of soda gave opium with

<sup>1</sup> Muller. *Archiv. der Pharmazie* (1914), 253, 4, pp. 280-293.

10.3 per cent. morphine. With the same pure race of poppy grown in the same season on our experimental area the extreme range of variation in the morphine content of opium from first lancements only was from 11.1 to 15.0 per cent. In the season 1919-20 these results were checked at Cawnpore. Around the edges of fields, owing to the land receiving poor cultivation, there are always a certain number of stunted plants which usually only produce one capsule. The smallest of these plants were selected and their terminal capsules lanced for opium. These plants were not as small as those grown specially in the previous season.

Four separate samples of opium collected yielded 11.6, 12.4, 12.3 and 12.7 per cent. morphine reckoned in the dry opium. The normal plants in the same field yielded opium containing 15 to 16 per cent. of morphine. Similar results were obtained in experiments in the Himalayas in 1919-20. The stunted plants from the edges of the fields produced opium with 10.3 per cent. and 7.3 per cent. morphine respectively in the two samples examined. The normal plants in the same field produced opium with 13 to 14 per cent. morphine.

Undoubtedly therefore stunted plants yield opium which is very low in morphine content, but the stunted plants examined by us were quite abnormal, and would never be used by the cultivators for lancing. These results contradict those of Wayne Arny<sup>1</sup>, who working on belladonna found that small plants produced the highest percentage of atropine.

Three of the four samples of opium collected from stunted plants at Cawnpore in 1919-20 whose morphine contents were 12.4, 12.3 and 12.7 gave the following respective percentages of codeine, viz., 4.35, 4.49 and 3.72.

Table XVI on page 116 gives the codeine content of the opium collected from normal plants on the manurial plots. These figures show little difference from those obtained for the codeine content of the opium from stunted plants and therefore in this respect codeine appears to behave differently from morphine.

#### THE INFLUENCE OF CLIMATE, SEASON AND WEATHER CONDITIONS.

*The Encyclopædia Britannica*, 11th edition, in the article on opium includes the following statement:—"It is a remarkable fact that the only Indian opium ever seen in England is an occasional sample of the Malwa sort, whilst the Government monopoly opium is quite unknown; indeed the whole of the opium used in medicine in Europe and the United States is obtained from Turkey. This is in some measure due to the fact that Indian opium

<sup>1</sup> Breeding for atropine. *Jour. of Heredity*, 1917, 3.

contains less morphine. It has recently been shown, however, that opium grown in the hilly districts of the Himalayas yields 50 per cent. more morphia than that of the plains and that the deficiency of morphia in the Indian drug is due, in some measure, to the long exposure to the air in the semi-liquid state which it undergoes. In view, therefore, of the probable decline in the Chinese demand the cultivators of the drug for the European market in the hilly districts of India and its preparation after the mode adopted in Turkey, *viz.*, by drying the concrete juice as quickly as possible, might be worthy of the consideration of the Government."

We have here a very definite statement that opium produced in the hills will contain more morphine than when grown in the plains. Enquiries in India, however, failed altogether to discover any evidence in support of it. Communications were then entered into with the writer of the article but they failed to establish any authority for the statement. The only references which the writer has been able to find bearing on this point are in the *Pharmacopœia of India*<sup>1</sup>. It is there stated that hill opium is superior to plains opium as a narcotic,<sup>2</sup> as it does not cause disagreeable symptoms such as headache, etc. A rough analysis for morphine, however, showed only 3 per cent. Pogson<sup>3</sup> states that an analysis of hill opium by Mactair showed that, when free from adulteration, it contains more morphine than the best Turkey opium and twice as much as the Government opium of Benares and Patna: the actual proportions however are not given. One can hardly quote the above old isolated references as evidence that hill opium is richer in morphine than plains opium. *The Encyclopædia Britannica*, statement may perhaps be traceable to a letter written by Col. Carruthers from Madras on May 6th, 1907 to the Secretary to the Director-General, Indian Medical Service. That writer remarked that the purple poppy (*Papaver glabrum*) is said to be cultivated in Kulu and opium derived from that district is undeniably good; it contains a good percentage of morphia and is in every way superior to ordinary Indian opium. Cushny<sup>4</sup> states that "Colder climates assist seed to produce a more powerful opium." The foregoing appears to be all the evidence so far as India is concerned which has any bearing on the effect of growing poppies in the hills on the morphine content of the opium produced. The writer considers that no suspicion of a case has been made out. In order that the point could be decided one has to use the same pure race of poppy and grow it in the same

<sup>1</sup> *Pharmacopœia of India*, by E. J. Waring, Appendix, page 437.

<sup>2</sup> MacGregor, *Diseases of North-Western Provinces, etc.*, Calcutta, 1843, pages 232-233.

<sup>3</sup> *Jour. Agri. Hort. Soc. Ind.*, 1863, Vol. XIII, p. 11 and page 9 App.

<sup>4</sup> *Pharmacology and Therapeutics*, 5th Ed., p. 212.

season in the hills as well as in the plains. Even then any difference observed may be due to different weather or soil conditions.

The writer has carried out experiments during the past four seasons on the effect of altitude on the morphine content of opium and these will be described later in this paper.

Rosenheim has recently contributed a paper in the *Biochemical Journal*<sup>1</sup> on "Biochemical changes due to environment." His results are concerned with the different amounts of pigments produced by the edelweiss inflorescence when grown in the Alps and near London respectively. He suggests that in the Alps the larger amount of pigment production is a natural protection against the larger amount of ultra violet rays present at the higher altitude since the pigments absorb these rays. He refers to work by Bonnier<sup>2</sup> who carried out experiments in Paris and on the Mont Blanc range and also by Kerner<sup>3</sup> who grew plants from seed at Vienna and at an altitude of 2,195 metres. Rosenheim summarizes their conclusions as follows:—"The main conclusions to be drawn from this work were that the whole habit of the plants grown at higher altitudes becomes more stunted and dwarfed than in the plains, the development of the subterranean parts was relatively greater and the internal structure of both stem and leaf was modified tending to lead to an increase of power of assimilation. Of the principal factors of the Alpine climate which gave rise to these changes the largest share is ascribed to the more intense illumination by day accompanied by low night temperature and a drier atmosphere. The writer has grown the same pure race of poppy in the plains of India and at various altitudes up to 6,500 ft. in the Himalayas. The climatic conditions under which the plant was grown were very different, as will be shown later in the paper. So far plants grown in the hills seem not to differ in appearance in any way from those grown in the plains.

Climate as such cannot be a predominating factor in morphine production by the poppy plant, for high morphine content opium has been produced in tropical, subtropical and temperate climates all over the world.

In Europe the Balkans are well known to produce high morphine content opium. In France, Bénard<sup>4</sup> at Amiens produced opium yielding 14.75 per cent. morphine reckoned on the dry material. Guibourt<sup>5</sup> in 1862 published a work on the composition of various kinds of opium grown in France. He found a

<sup>1</sup> *Biochem. Jour.*, XII, 4, December 1918, pp. 283-9.

<sup>2</sup> *Bull. Soc. Bot.*, 1888, 35, 436. *Ann. Sci. Nat. (Bot.)*, 1894, ser. 7, 20, 217.

<sup>3</sup> *The Natural History of Plants* (London), 1894.

<sup>4</sup> Descharmes. *Comptes Rend.*, 1854, II Series, 751.

<sup>5</sup> Wiesner. *Die Rohstoffe des Pflanzenreiches*, p. 407.

high morphine content varying from 12.1 to 22.9 per cent. calculated on the dry matter. The latter is the highest figure on record.

Quite a number of analyses of opium produced in Germany<sup>1</sup>, e.g., Schleswig, Wurttemberg, and Bohemia, have given 9-10 per cent. 13-15 per cent. and 11-12 per cent. of morphine in opium respectively. Biltz<sup>2</sup> in 1829 and 1830 shewed that high morphine content opium could be produced in both North and South Germany. Desaga<sup>3</sup> in Karlsruhe produced opium containing 16½ per cent. morphine in its dry matter. For other German analyses see Merck<sup>4</sup>, Harz<sup>5</sup>, Jobst<sup>6</sup>, Schwendt<sup>7</sup> and Thoms<sup>8</sup>, all of whom grew opium of high morphine content.

In Sweden<sup>9</sup>, at Upsala, Almquist produced opium containing 12 per cent. morphine.

Turning to America one finds that the U. S. Department of Agriculture has in recent years experimented in poppy cultivation for opium production in the Southern States. The results were recorded by E. Weschke<sup>10</sup> in a long article in the *Pacific Medical Journal* in 1905. The average morphine content of the opium was 15.28 per cent.

In Africa it is known that Egypt can produce high morphine content opium. Algiers<sup>11</sup> reports 14 per cent. morphine in opium. German East Africa<sup>12</sup> has also experimented with poppy cultivation and opium produced there in 1905 contained 14.30 per cent. morphine reckoned on the dry matter. Aubergier<sup>13</sup> found up to 17.8 per cent. morphine in Algerian opium.

Australia has produced opium containing 10 per cent. morphine<sup>14</sup> and 11.5 per cent. morphine<sup>15</sup>. It would seem however that in Australia the successive landings are all mixed and therefore these figures are probably low<sup>16</sup>.

<sup>1</sup> Wiesner. *Die Rohstoffe des Pflanzenreiches*, p. 497.

<sup>2</sup> Thoms. *Ueber Mohnbau und Opiumgewinnung*, Berlin, 1907, p. 3.

<sup>3</sup> *Jahresber d. Pharm.* 1868, S. 113

<sup>4</sup> „ „ 1872, S. 192

<sup>5</sup> „ „ 1869, S. 116

<sup>6</sup> „ „ 1868, 1869

<sup>7</sup> „ „ 1870, S. 189

Through Thoms, *loc. cit.*

<sup>8</sup> Thoms. *loc. cit.*

<sup>9</sup> *Jahresber d. Pharm.* 1873, S. 141. Through Thoms. *loc. cit.*

<sup>10</sup> *Pharm. Jour. and Trans.*, 1895, p. 493, and *Deutsch-Amerikanische Apotheker Zeitung*, 1905, S. 106.

<sup>11</sup> Thoms. *Ueber Mohnbau und Opiumgewinnung*, Berlin, 1907, p. 3.

<sup>12</sup> K. Braun. *Der Pflanze Ratgeber für tropische Landwirtschaft unter Mitwirkung des Biologisch Landwirtschaftlichen Institute Amani*, 29th July, 1905. Through Thoms. *loc. cit.*

<sup>13</sup> *Ann. Chim. Phys.* (iii) 20, 303. Through Thorpe's *Dict. of Applied Chemistry*.

<sup>14</sup> Wiesner. *Die Rohstoffe des Pflanzenreiches*, p. 407.

<sup>15</sup> Thorpe. *Dict. of App. Chem.*, vol. IV, p. 19.

<sup>16</sup> Thorpe. *loc. cit.*, p. 18.



Asia Minor opium and Persian opium are well known to be of high morphine content.

Japanese<sup>1</sup> opium may contain from 10-20 per cent. morphine and Chinese<sup>2</sup> opium has yielded 11.27 per cent. morphine.

Finally, our own work has shown that India can produce opium of as high morphine content as any country in the world. The writer found the opium of a pure race of poppy isolated by Mr. Leake and grown at Cawnpore to contain over 20 per cent. morphine in the dry matter.

It seems that Tunmann<sup>3</sup> is correct in the following statement that "the amount of sap and its alkaloid content are not dependent on climate since one can grow just as good opium in Sweden, China or Zambesi as in Asia Minor."

If we turn to certain of the other alkaloids present in opium however, e.g., narcotine, codeine, papaverine, thebaine and narceine, we shall find that there is a good deal of evidence to show that opium of various countries may show peculiarities with respect to them.

As regards narcotine, Indian and Egyptian opiums are stated to be generally richer in this alkaloid than most other opiums.<sup>4</sup> According to Uyeno<sup>5</sup> Japanese opium also contains large amounts of narcotine, e.g., 7 to 11 per cent. Various workers have reported from time to time French opium which contained no narcotine. Thus Pelletier,<sup>6</sup> working on opium grown at Eyres, Department des Landes, found no narcotine in 60 gm. of it. Descharnes<sup>7</sup> reports that in opium from the north of France he found neither narcotine, thebaine, nor narceine in any appreciable quantity.

Guibourt<sup>8</sup> working with opium from Amiens produced by *Papaver somniferum* L. var. *nigrum* found no narcotine. Van Itallie and Kerbosch<sup>9</sup> were able to check this result 50 years later on some of the sample which had been kept in a collection and they also found no narcotine. They however found an alkaloid somewhat similar to narcotine which so far they have been unable to identify.

Turning to codeine, Indian opium appears generally much richer in this alkaloid than Turkish opium. The writer has often found 4 per cent.

<sup>1</sup> Uyeno, Beckurts. *Jahresber*, 1892, p. 122, and also private information.

<sup>2</sup> *Pharm. Jour. and Trans*, 1895, p. 493.

<sup>3</sup> *Pflanzenmicrochemie*, Berlin, 1913.

<sup>4</sup> *Allen Comm. Org. Analysis*, vol. VI, p. 409.

<sup>5</sup> Beckurts. *Jahresber*, 1892, p. 141.

<sup>6</sup> *Journ. de pharm. et des Sc. access.*, 21, 570, 1835. Through van Itallie and Kerbosch.

<sup>7</sup> De l'opium indigène extrait du pavot œillette. *Extr. du mem. de l'acad. des Sc. du Dep. de la Somme*, 1862, p. 52.

<sup>8</sup> *Memoir sur le dosage de l'opium*, Paris, 1862, p. 52.

<sup>9</sup> *Archiv. der Pharmazie*, 1911, 248, p. 61.



codeine in Indian opiums and never less than 1·8 per cent. The Imperial Institute<sup>1</sup> found the codeine content of Indian opiums to vary from 2·4 per cent. Persian<sup>2</sup> opium is said to contain an average of  $2\frac{1}{2}$  per cent. of that alkaloid.

Van Itallie and Kerbosch<sup>3</sup> determined the presence or absence in opium of various sources of the chief opium alkaloids, *viz.*, morphine, narcotine, papaverine, thebaine, codeine and narceine. They worked with opiums from Asia Minor, Bengal, Malwa, Patna, China, America, France, Persia and Egypt. All these alkaloids were found present in all the opium except papaverine which was absent in Bengal, Patna and Benares opium. It is remarkable that papaverine was found in Malwa opium which is also Indian. The authors in discussing the characters of the poppies grown in various countries consider that the absence of papaverine cannot be due to the growth of a different kind of plant since it seems that Bengal, Persian, Egyptian and other opiums are all produced from *Papaver somniferum* var. *album*. In this connection it is interesting to note that the poppies grown in Malwa have coloured flowers. Those grown in British India are on the other hand exclusively white flowered. The authors state that they are investigating the reason for the absence of papaverine from certain samples.

Allen's<sup>4</sup> "Commercial Organic Analysis" states that "the variety of poppy cultivated in Asia Minor is said to be the *black* which usually has purple flowers and black though occasionally white seeds. It is said to be usually richer in morphine than that from the white flowered and white seeded poppy which is rich in narcotine and appears to be the only kind cultivated in Egypt, Persia, India, China and Japan."

Van Itallie and Toorenburg<sup>5</sup> report that the opium of *Papaver somniferum* var. *nigrum* contains no narcotine.

The writer hopes to throw light on this interesting question, owing to the possession of opium produced from many hundreds of pure races of poppy and it is proposed as time permits to examine these for codeine and narcotine content.

A certain amount of work has been done on the effect of climate on the alkaloidal content of certain other medicinal plants and it will perhaps be worth while summarizing it here.

<sup>1</sup> *Bull. Imp. Institute*, vol. XIII, 1915, pp. 507-546.

<sup>2</sup> *Bull. Imp. Institute*, vol. XIII, 1915, p. 510; and *Enc. Brit.*, 11th Edn., Article on opium p. 132.

<sup>3</sup> *Archiv der Phar.*, 1911, 248, p. 613.

<sup>4</sup> Vol. VI, p. 407, Footnote.

<sup>5</sup> *Pharm. Weekblad.*, 1915, 52, p. 1601

Dunstan<sup>1</sup> reports climatic effects in the case of *Hyoscyamus noticus* and *Datura stramonium* grown in India and Egypt.

Schmidt<sup>2</sup> and Kirchner recorded a great variation of the ratio of hyoscyamine and hyoscyne in *Datura arborea* grown in Germany and India.

Brindejonc<sup>3</sup> working with roots of *Eschscholtzia Californica* grown in Brittany obtained results quite different from those recorded from plants grown in other localities. His roots contained only one alkaloid, ionidine, present to the extent of 0.25 per cent.

It is regarded as a notable instance of the profound difference that soil and climate may effect in the constituents of plants.

Chevalier<sup>4</sup> gives the following analyses of belladonna from various countries:—

Country	Per cent. of alkaloids
Italy	0.058—0.187
Austria	0.251—0.372
France	0.300—0.450

Winterstein<sup>5</sup> and Trier state that climate is able to bring about important changes in the alkaloid content of plants, *e.g.*, with conium and cinchona.

It would therefore seem that there is a general tendency to assume that the alkaloidal content of medicinal plants varies according to the country in which they are grown.

The evidence in support of this idea does not seem to the writer very strong. There are so many factors involved besides the geographical one, *e.g.*, soil, race of plant, and weather conditions and incidentally the error in sampling and the method of analysis used. The writer has been unable to find in the literature any convincing experiments on the point. Such experiments are of course difficult to make. One needs to work with the same pure race of plant and the experiments have to be carried out on a large scale and over a number of seasons, and the conditions of harvesting of the produce must be identically the same. Even then one is bound to have different soil conditions, perhaps different sowing and harvesting seasons and so on. As regards opium we have a case in which it has been definitely stated that the

<sup>1</sup> *Year book of Pharmacy*, 1899, p. 143; 1901, p. 70; 1903, p. 560; 1906, p. 97

<sup>2</sup> Carr and Reynolds *Pharm. Jour.* (4), 26, 513.

<sup>3</sup> *Bull. Soc. Chim.*, 1911, 9, p. 97.

<sup>4</sup> *Comptes Rend.*, 1910, 150, p. 345.

<sup>5</sup> *Die Alkaloide*, Berlin, 1910, p. 264.

plains of India cannot grow as high morphine content opium as the hills and that India produces opium of lower morphine content than Turkey. The writer's experiments show that these are fallacies : Indian opium is of lower morphine content than the Turkish because the system of harvesting is different in the two countries.<sup>1</sup> The climate in the case of opium has certainly little to do with the matter.

Although it has been shown that high morphine content opium can be grown in countries possessing widely different climates, yet there is much evidence which goes to show that sudden changes in weather conditions may affect the drug-yielding power of plants. Indian opium cultivators state that cloudy weather or east winds diminish the out-turn of opium and also its quality. Under these circumstances much *pasewa* is produced. *Pasewa* is a thick black liquid which darkens the colour of the opium and which is difficult to separate from the opium. Opium containing *pasewa* is paid for at lower rates by Government and hence in bad *pasewa* years the cultivators suffer heavy losses. The nature and the origin of *pasewa* is practically unknown. In work on palms the writer has shown that the flow of palm juice is also affected by weather conditions. East winds and cloudy weather produce juice which ferments rapidly. Cold nights are associated with heavier yields of juice.

Muller<sup>2</sup> finds that the alkaloidal content of poppy plants is materially affected by weather conditions, the amount decreasing in wet or cloudy weather and increasing in sunny weather.

Mitlacher<sup>3</sup> and Hayes publish some inconclusive work on the effect of climatic conditions on morphine production in opium.

Thoms<sup>4</sup> published figures showing the effect of season on the out-turn and composition of opium from the same variety of seed grown in the same locality for two successive seasons. He grew Smyrna and German poppies. The yields of opium in the case of the latter were far higher in 1905 than in 1906. The morphine content of the opium, however, seems to have been very

<sup>1</sup> Annett, Sen and Singh. *Mem. Dept. Agri. India (Chemical Series)*, vol. VI, no. 1. (pt. III).

<sup>2</sup> *Archiv der Pharm.*, 1914, 252, pp. 280-293.

<sup>3</sup> *Pharm. Post. Nouveaux Remèdes*, 1914, 31, 121 (through *Year Book of Pharm.*, 1914, p. 201.)

<sup>4</sup> *Loc. cit.*

little different in the two years except in the case of the Smyrna poppies. The table summarizes the results:—

*Morphine per cent. on dry opium.*

Variety	1906	1905
Smyrna black . .	10.70	13.40
German blue-seeded .	10.59	11.12
German white-seeded .	10.87	10.84

The season 1906 was warm and sunny and there seemed no reason why yields should have been low. The capsules and plants seemed just as big as in the previous year, yet their yield of latex was less. Many capsules yielded no latex at all.\* Since pure races of poppy were not employed one cannot put much reliance on these results.

There is also a certain amount of work bearing on the effect of varying weather conditions on the drug production by other plants. F. H. Carr<sup>1</sup> reports experiments showing the relation of weather conditions to alkaloidal production in belladonna. The table summarizes the results:—

Year	Percentage of alkaloids in stem and leaf	Total hours sunshine May 1st to June 30th	Rainfall (same period) inches
1905 . .	0.38	387	5.48
1906 . .	0.54	361	3.86
1907 . .	0.34	290	3.54
1908 . .	0.48	387	5.44
1910 . .	0.61	360	4.08
1911 . .	0.59	404	3.62
1912 . .	0.68	Unusually dry and sunny season.	

Carr concludes from these results that the highest percentages of alkaloids were obtained in the most sunny and dry seasons; while the low percentages found in 1905 and 1907 are explained by the heavy rainfall in the former and the lack of sunshine in the latter season.

\* *Note.* It is a fairly common experience in India to find capsules which yield no latex on lancing. These are generally distinguished by a reddish tint.

<sup>1</sup> *Inter. Com. App. Chem. Chem. and Drug.*, 1912, 81, p. 432. Effect of cultivation on alkaloidal content of belladonna.

The alkaloidal content of belladonna would appear to vary considerably from year to year according to the following abstract<sup>1</sup> :—

“As regards belladonna root, especially that imported from the continent, a root containing 0·5 per cent. of alkaloids was at one time obtainable without difficulty, and 0·6 per cent. was occasionally met with. Then, for some years, it was difficult to find any containing 0·4 per cent., many samples only yielding about 0·2 per cent. Recently, there has been some little improvement. Whether the variations are due solely to the seasons or whether there are other conditions affecting the constituents of the drugs is a subject inviting investigation. The following analyses of dried belladonna leaf and stem grown on the same plot in three successive years indicate a big seasonal variation :—

1905	..	..	0·36	Per cent. total alkaloids
1906	..	..	0·66	” ” ”
1907	..	..	0·53	” ” ”

The resin of jalap seems to have undergone a similar seasonal variation.”<sup>2</sup>

Chevalier<sup>3</sup> records results of experiments with broom tops gathered month by month from the same locality throughout the year. The results are expressed in grm. of sparteine sulphate per kilogram of dried plant.

Month	Grm. of sparteine sulphate per kilogram of dried plant
January . . . . .	0·02
February . . . . .	4·15
March . . . . .	6·80
April ] . . . . .	3·25
May . . . . .	4·32
June . . . . .	3·27
July . . . . .	3·00
August . . . . .	2·33
September . . . . .	3·58
October <sup>1</sup> . . . . .	4·27
November . . . . .	4·75
December . . . . .	4·07

<sup>1</sup> *Year Book of Pharmacy*, 1910. Presidential Address, p. 314.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> *Comptes Rendus*, 1910, 150, p. 1068.

According to Carr<sup>1</sup> these variations would appear to correspond to changes during the flowering and growing period.

Jowett and Potter<sup>2</sup> have recorded a similar seasonal variation in the amount of salicin in the bark of *Salix nigra*.

Farr and Wright<sup>3</sup> in experiments on conium also shew the great effect of season in this case.

Burmam<sup>4</sup> has carried out a systematic determination of the chief active principles of certain plants for four successive years, and his results indicated that plants grown in the same locality have undergone a marked deterioration in potency. This was attributed to the inclement weather during the last two summers which gave low temperatures and less sunshine. Rain as such is stated not to affect the amount of active principle except so far as its presence generally coincides with cloudy weather.\* Burmann's results are:—

Plant	Active principle determined	YEAR			
		1907 %	1908 %	1909 %	1910 %
<i>Aconitum napellus</i>	Aconitine	0.104	0.100	0.042	0.054
<i>Belladonna</i>	Atropine	0.094	0.082	0.045	0.046
<i>Colchicum</i> seeds	Colchicine	0.190	0.160	0.144	0.148
<i>Digitalis ambigua</i>	Digitoxine	0.134	0.120	0.067	0.069
" <i>purpurea</i>	Digitoxine	0.078	0.063	0.033	0.037
<i>Ergot</i>	Cornutine	0.300	0.260	0.250	0.220

Burmam<sup>5</sup> published further data shewing that the amount of active principles formed in different years is in direct ratio to the temperature. He worked with *Colchicum*, *Digitalis ambigua*, *D. purpurea*, *Aconitum napellus* and *Atropa belladonna*. He gives a graph shewing the mean temperature for each year from 1907 to 1911, and he also plots out the amount of alkaloids or glucosides found in the above plants. In all cases these follow the temperature, being lowest in the cold weather of 1909 and highest in the warm year of 1911.

\* Cloudy weather adversely affects sap flows and latex flows. See the writer's work on palms. *Mem. Dept. Agri. India, Chem. Ser.*, vol. II, no. 6.

<sup>1</sup> *Pharm. Jour.* (4) 26, 543. Carr and Reynolds.

<sup>2</sup> *Year Book of Pharm.*, 1902, p. 482.

<sup>3</sup> *Year Book of Pharm.*, 1904, p. 70.

<sup>4</sup> *Schweis. Woch. Chem. Pharm.*, 1911, 49, p. 6.

Annual Variation in Potency of Medicinal Plants. (Through Abs. in *Year Book of Pharm.*, 1911.)

<sup>5</sup> Influence of temperature on formation of alkaloids and glucosides in plants. *Schweis. Woch. Chem. Pharm.*, 1913, 51, p. 117. (Through *Year Book of Pharm.*, 1913, p. 3.)



Unger<sup>1</sup> found that belladonna leaves grown in the shade contained 0.35 per cent. of total alkaloids and 15.07 per cent. of ash in dry matter.

A sample grown in the sun gave 0.4 and 13.34 per cent. respectively.

#### EXPERIMENTAL.

##### 1. *Effect of climate.*

During the past seasons 1916-20, for the purpose of these experiments poppies have been grown at various stations in the plains of the United Provinces and also at three different stations in the Himalayas. The seed used was that of two pure races of poppy which had been isolated by Mr. H. M. Leake, Economic Botanist to Government, United Provinces.

The seed used in 1916-17 was a pure race of the variety known as Katela. Unfortunately, only one set of analyses was made of the opium produced at Cawnpore, but at the same time this was the total produce of a fair-sized area, viz., about one-fourth of an acre. During the same season seed of the same pure race had been grown in the Himalayas at three stations, viz.; Douglas Dale (altitude 4,000 ft.), Chaubattia (altitude 6,500 ft.), and Almorah (altitude 4,500 ft.).

The seed sown in 1917-18, 1918-19 and 1919-20 was another of Mr. Leake's pure races, which has been designated K 55. The writer has grown this seed under all sorts of experimental conditions and is in possession of much data bearing on the morphine content of the opium produced by it, and on the yield of opium per capsule. He is thus in a good position to judge of any changes brought about in the composition or yield of the opium by growing the seed in the hills.

In 1917-18 about 5 acres were grown at Cawnpore from this seed, and 27 acres grown in the Rae Bareli District, 80 miles north-east of Cawnpore. Small areas were also grown at Mianganj and Ankin in the Fatehgarh District, 40 miles west of Cawnpore. In the Himalayas, it was grown at the three stations above mentioned, viz., Douglas Dale, Chaubattia and Almorah.

In 1918-19, seed of the same pure race was grown at Cawnpore and Rae Bareli in the plains and at Douglas Dale in the Himalayas. The respective areas were 5 acres, 30 acres and  $\frac{1}{2}$  acre at the three places.

In 1919-20, seed of the same pure race was grown at Cawnpore (5 acres), Rae Bareli (30 acres), Etawah and Fatehgarh in the plains, and at Douglas Dale in the Himalayas.

##### *Climate of the various centres.*

The climate of the plains stations Cawnpore, Ankin, Mianganj, Fatehgarh, Etawah and Rae Bareli may be taken as being more or less identical. The

<sup>1</sup> *Apoth. Zeit.*, 1912, 27, p. 763. Alkaloidal value of belladonna grown in light and shade.

seed is usually sown in November and the opium is harvested some time in March or perhaps at the end of February. The following data give an indication of the nature of the weather experienced at Cawnpore during the cold weather 1916-17.

*Table showing temperature and rainfall at Cawnpore. Poppy season, 1916-1917.*

Month	Mean maximum temperature	Mean minimum temperature	Maximum temperature	Minimum temperature	Rain-fall	No. of rainy days
October 1st portion	89	72	95	69	1.93	4
" 2nd portion	90	64	94	52	..	..
November 1st portion	86	52	89	46	..	..
" 2nd portion	78	49	83	44	2.45	2
December 1st portion	76	45	79	42	..	..
" 2nd portion	73	41	78	36.5	..	..
January 1st portion	74	40	80	37	..	..
" 2nd portion	78	48	83	42.5	0.37	2
February 1st portion	73	48	81	41	0.96	2
" 2nd portion	85	51	89	46	..	..
March 1st portion	87	53	97.5	48	0.12	2
" 2nd portion	94	60	101	53	..	..

At the Himalayan stations the sowing time was more or less the same as in the plains, but the growing period is rather longer, the opium not being harvested until April and early May. The weather is also much colder on the average and much more stormy. Rain is frequent and at Chaubattia in 1916-17 the crop was at one stage under snow. The following table summarizes the weather conditions at the three Himalayan stations for 1917-18 :—

Month	DOUGLAS DALE ALTITUDE 4,000 FEET			ALMORAH (SITOLI) ALTITUDE 4,500 FEET			CHAUBATTIA ALTITUDE 6,500 FEET		
	Mean maximum temperature	Mean minimum temperature	Rain-fall	Mean maximum temperature	Mean minimum temperature	Rain-fall	Mean maximum temperature	Mean minimum temperature	Rain-fall
October . . . .	..	..	4.25	70.8	56.6	3.69	60.3	49.7	3.97
November . . . .	..	..	0.03	65.9	48.0	0.00	54.2	43.2	0.00
December . . . .	..	..	1.10	63.2	42.1	0.90	41.5	32.9	1.02
January . . . .	..	..	0.44	57.5	39.7	1.04	41.3	37.0	0.73
February . . . .	..	..	0.00	63.0	43.8	0.00	48.9	36.8	0.00
March . . . .	..	..	1.31	68.0	49.5	1.30	56.3	39.6	2.03
April . . . .	..	..	2.07	72.4	53.2	2.36	Not available		2.04
May . . . .	..	..	2.75	81.1	65.9	1.63	Not available		2.17

The weather conditions at the various experimental centres are therefore seen to be very different. It is unfortunate that the temperature records of

Douglas Dale are unobtainable. The results of the experiments are set out in the following table:—

*Table showing effect of climate or locality on the morphine content of the opium. Produce of first incision of capsules only.*

No. of samples examined	Locality	PERCENTAGE OF MORPHINE CALCULATED ON OPIUM DRIED AT 100°C.	
		Range of variation	Average
1916-17.			
10	Sitoli (altitude 4,500 ft.) . . . . .	10·9—13·9	12·7
1	Douglas Dale (altitude 4,000 ft.) . . . . .	....	10·6
1	Chaubattia (altitude 6,500 ft.) . . . . .	....	10·3
1	Cawnpore . . . . .	....	11·1
1917-18.			
91	Cawnpore . . . . .	11·8—15·8	14·0
1	Fatehgarh (Ankin) . . . . .	....	15·1
2	„ (Mianganj) . . . . .	12·6—13·1	12·8
5	Rae Bareli . . . . .	11·2—13·4	12·2*
11	Douglas Dale (altitude 4,000 ft.) . . . . .	11·3—16·7	13·3*
1	Sitoli (altitude 4,500 ft.) . . . . .	....	10·0
2	Chaubattia (altitude 6,500 ft.) . . . . .	16·1—16·3	16·2
1918-19.			
119	Cawnpore . . . . .	10·5—15·8	12·6
12	Rae Bareli . . . . .	9·2—12·7	11·1
8	Douglas Dale (altitude 4,000 ft.) . . . . .	9·3—12·4	11·1
1919-20.			
71	Cawnpore . . . . .	14·0—17·7	15·4
8	Rae Bareli . . . . .	11·5—15·0	13·1
1	Fatehgarh . . . . .	....	16·6
2	Etawah . . . . .	16·1—17·6	16·8
3	Douglas Dale (altitude 4,000 ft.) . . . . .	13·2—15·3	14·0

Dealing first with the 1916-17 experiments it will be seen that numbers of analyses of the opium grown at Sitoli were made, the morphine content in the dry opium varying from 10·9 to 13·9 per cent. Only one analysis is available from each of the other three centres. The morphine content of the opium produced at Cawnpore was lower than that from Sitoli, and higher than that from the other two hill stations. Hence one can hardly say that the hill opium proved richer in morphine than the plains opium.

The 1917-18 experiments were, however, much more complete and gave a much better basis for judging of any possible climatic effect. Thus for Cawn-

\* These samples were mixtures of first and second lancings and would therefore be lower in morphine content than first lancings only.

pore the table records the results of analyses of 91 samples of opium, all first lancements, taken from different fields of the same pure race scattered over an area having a radius of about quarter of a mile. The extreme variations in morphine content were from 11.8 to 15.8 per cent., most of the figures running from 14 to 15 per cent. The actual average of all the Cawnpore analyses is just 14 per cent.

From Douglas Dale there are 11 analyses of samples taken from different parts of an area of about 1 acre. The morphine content of the samples varied from 11.3 to 15.7, a rather larger range than at Cawnpore with an actual average for the 11 samples of 13.3 per cent. morphine. So that there was practically no difference in the morphine content of the opium grown at Cawnpore and at Douglas Dale.

Sitoli unfortunately only provided one sample, which only gave 10 per cent. morphine.

From Chaubattia there are figures for two samples of opium, and both samples had a morphine content rather better than the best Cawnpore opium.

As regards the remaining plains stations a sample of opium grown at Ankin yielded 15.1 per cent. morphine, practically the same figure as from Cawnpore. The opium from Mianganj was unfortunately the product of the first two lancements, but it gave in its two samples 13.9 and 12.6 per cent. morphine, which figures agree fairly well with what one would expect. The five samples produced at Rae Bareli were also the product of the first and second lancements, and they varied in their morphine content from 11.3 to 13.45 per cent., with a general average of 12.2 per cent., which figure is also about what one would expect.

On the whole, therefore, it appears that the morphine content of the opium is not appreciably different whether produced in the hills or the plains.

As regards the yield of opium per 1,000 capsules, the following results were obtained in 1917-18, taking the product of the first lancements only into consideration :—

Cawnpore 97.2 to 15.0 grm. with an average of 47 grm. per 1,000 capsules.

Douglas Dale 40.2 to 16.4 grm. „ „ 27 „ „ „

Sitoli 16.4 grm. from one sample.

Chaubattia 16.1 and 16.3 grm. for two samples.

The yield of opium per capsule at the first lancing would therefore appear to be lower in the hills than in the plains.

However, the plant seems to behave very differently in the hills from what it does in the plains. In the plains the yield at each successive lancing

per 1,000 heads shows a progressive fall. On the other hand, each of the first four lancements gives, in the hills, about the same out-turn, and frequently the produce of the second and third lancements is each bigger than that of the first lancing. As a result of this the total out-turn of opium in the hills per 1,000 capsules was fully equal to what was obtained in the plains. The writer ascribes the high yield of opium at the later lancements in the hills to the fact that the cooler weather at harvesting time does not dry up the capsules so rapidly as in the plains where the harvesting is carried out during a rapidly rising temperature, with frequent occurrence of hot, dry west winds.

The figures for 1918-19 and for 1919-20 only include the results from one of the Himalayan stations, *viz.*, Douglas Dale. The morphine content of the opium produced there differs from that grown at Cawnpore to an even less extent than does that grown at Rae Bareli, a plains station within 80 miles of Cawnpore.

## 2. *The influence of seasons.*

The same pure race of poppy has been grown at Cawnpore on the same farm during the past four seasons. In 1916-17 the area grown was 0.5 acre and this was divided into 16 plots and the opium of each analysed separately. In 1917-18 and 1918-19 the area grown was just under 6 acres and in 1919-20 it was  $1\frac{1}{2}$  acres.

The same pure race has been grown on a much larger scale during three successive seasons at Rae Bareli, a plains station 80 miles from Cawnpore. It has also been grown during three successive seasons at Douglas Dale, 4,000 feet up in the Himalayas. The results are set out in the table:—

Season	Area under experiment in acres	No. of samples analysed	PERCENTAGE OF MORPHINE CALCULATED ON OPIUM DRIED AT 100°C.	
			Range of variation	Average
<i>Cawnpore.</i>				
1916-17 . . . . .	0.5	16	11.7-14.7	12.9
1917-18 . . . . .	6.0	91	11.8-15.8	14.0
1918-19 . . . . .	6.0	119	10.5-15.8	12.6
1919-20 . . . . .	1.5	71	14.0-17.7	15.4
<i>Rae Bareli.</i>				
1918-19 . . . . .	20	12	9.2-12.7	11.1
1919-20 . . . . .	30	8	11.5-15.0	13.1
<i>Douglas Dale.</i>				
1917-18 . . . . .	0.5	11	11.3-16.7	13.3
1918-19 . . . . .	0.5	8	9.3-12.4	11.1
1919-20 . . . . .	0.5	3	13.2-15.3	14.0



The figures indicate that there is a distinct variation in morphine content from season to season. Thus 1918-19 seems to have been a poor season for morphine production. 1919-20 has given opium of distinctly higher morphine content at Cawnpore than any of the preceding seasons, and it seems to have been a good season both for Rae Bareli and Douglas Dale.

A number of other pure races have been grown at Cawnpore during two and in some cases three successive seasons. The morphine content of the dried opium of the first lancements is set out in the table.

No. of pure race	PERCENTAGE OF MORPHINE IN DRY OPIUM			No. of pure race	PERCENTAGE OF MORPHINE IN DRY OPIUM		
	1917-18	1918-19	1919-20		1917-18	1918-19	1919-20
29	13.9	11.7	....	343	19.0	12.8	15.5
35	15.1	12.9	....	344	18.7	14.1	....
50	16.9	13.6	16.7	347	19.3	14.0	16.9
127	17.3	14.7	....	1592	13.1	10.7	....
172	12.8	10.6	....	1596	15.6	15.8	17.0
237	17.6	15.2	....	1600	16.0	14.0	....
248	18.6	16.4	....	1604	13.1	11.5	....
251	16.8	17.3	16.8	1605	15.2	12.2	....
269	14.2	14.1	....	1637	16.6	11.7	....
278	15.7	16.0	....	1641	15.6	12.9	15.1
279	15.4	14.6	....	1644	14.8	10.6	16.8
287	19.6	14.8	15.5	1645	14.9	11.4	15.4
290	16.4	15.4	....	1650	14.3	12.3	....
294	14.3	13.6	....	1669	18.7	12.9	....
297	13.7	15.1	....	1682	11.1	7.9	....
312	14.6	12.3	....	1875	14.8	11.9	....

These figures again indicate a seasonal influence. 1918-19 here again shows up as a bad morphine year, whereas 1919-20 appears to have been much better in this respect. In this connection the writer has been informed that there is a certain amount of variation from season to season in morphine content of the opium produced in Asia Minor.

### 3. *The influence of weather conditions, 1917-1918.*

An experiment on a large scale was devised in order to try to trace any connection there might be between temperature and other weather conditions and yield or morphine content of the opium produced. A continuous record of temperature was kept by means of a thermograph. Observations were also made of barometric and humidity conditions, but furnished no results worthy of consideration here. Rainfall was recorded and also the direction of the wind, amount of cloud, etc. Opium was collected daily from capsules which were considered by the cultivators to be in the correct stage for lancing.



Lancings were carried out daily from 28th February to 26th March 1918. A field was taken which measured 0.5 acre. It had been sown with the seed of a pure race of poppies. Every day the cultivators were told to select about 1,400 terminal capsules which they considered ready for lancing. The selected capsules were marked each day with a cloth label of a distinctive colour. Each lot of capsules received a second and a third lancing at 48 hour intervals. Thus, on 28th February 1918, the capsules selected were marked with white cloth labels and lanced that afternoon. On the 1st March more capsules were selected and labelled with pink labels and lanced. On the 2nd March more selected capsules (labelled blue) were lanced and also a second lancing was made of the capsules labelled white which had been first lanced on the 28th February. On the 3rd March a further lot of fresh capsules were labelled with blue labels and lanced. On that day also a second lancing was made of the capsules labelled pink which had been first lanced on the 1st. On the 4th March more capsules were labelled with red labels and lanced, and the second lancing of the blue-labelled capsules was also carried out, in addition to a third lancing of the white-labelled capsules. The white-labelled plants were pulled up after the next day's opium collection and counted and examined to see that they had each received three lancings. This procedure was continued throughout the experiment, each group of plants being pulled up and counted after they had been lanced three times. The numbered plants in each group were also counted daily at the time of opium collection. So that each group of plants was counted three times over. Once plants with a certain coloured label were removed, these labels could of course be again put on to another series of plants. Only terminal capsules were selected on these experiments owing to the low morphine content of the opium from lateral capsules.<sup>1</sup> The field was marked off into 10 plots to simplify counting and the finding of the various marked plants, which, even with distinctive coloured labels, was at times difficult. Moreover, the opium of each set of plants was collected in two lots, so that there could be duplicate determinations for morphine content. Towards the end of this experiment this was not always done owing to the small yield of latex obtained. The determination of morphine content of the duplicate samples of opium collected agreed so well in practically every case that the average figure only has been inserted in the table. The method of selection of the plants tended to rule out any variations in soil conditions. All the figures obtained are set out in the following table, and in the curves on Chart VI.

<sup>1</sup> See p. 62.

Yield of opium per 1,000 capsules and its morphine content recorded daily throughout duration of opium harvest, 1917-1918.

FIRST LANCING					SECOND LANCING					THIRD LANCING				
Date of lancing	No. of cap-sules lanced	Grm. dry opium	Dry opium per 1,000 cap-sules	Mor-phine per cent. on D. M.	Date of lancing	No. of cap-sules lanced	Grm. dry opium	Dry opium per 1,000 cap-sules	Mor-phine per cent. on D. M.	Date of lancing	No. of cap-sules lanced	Grm. dry opium	Dry opium per 1,000 cap-sules	Mor-phine per cent. on D. M.
28-2-18	1400	72.9	52.1	14.9	2-3-18	1371	48.6	35.4	10.2	4-3-18	1353	25.1	18.5	7.0
1-3-18	1402	87.1	62.1	14.5	3-3-18	1392	39.9	28.7	9.9	5-3-18	1384	21.3	15.4	6.3
2-3-18	1400	84.4	60.3	14.9	4-3-18	1400	46.52	33.2	9.4	6-3-18	1392	18.9	13.6	6.3
3-3-18	1360	72.36	53.1	14.9	5-3-18	1351	43.02	31.9	9.8	7-3-18	1349	14.8	10.9	6.2
4-3-18	1400	84.2	60.1	14.6	6-3-18	1318	43.39	32.9	9.2	8-3-18	1312	11.9	9.0	6.6
5-3-18	1370	83.7	61.1	14.5	7-3-18	1370	39.7	28.9	9.5	9-3-18	1370	11.5	8.4	7.6
6-3-18	1400	90.3	64.5	13.9	8-3-18	1400	34.8	24.9	8.9	10-3-18	1400	10.5	7.5	6.4
7-3-18	1400	75.3	53.7	14.2	9-3-18	1400	30.8	22.0	10.4	11-3-18	1400	13.5	9.6	6.1
8-3-18	1400	71.9	51.3	13.6	10-3-18	1400	32.2	23.0	11.0	12-3-18	1400	11.4	8.1	9.2
9-3-18	1390	17.2	50.4	14.0	11-3-18	1390	29.8	21.5	8.8	13-3-18	1390	9.0	6.5	7.7
10-3-18	1280	55.2	43.2	14.2	12-3-18	1280	27.3	21.3	10.1	14-3-18	1278	10.7	8.3	7.5
11-3-18	1300	58.5	45.4	14.1	13-3-18	1300	24.4	18.7	11.6	15-3-18	1300	10.0	7.7	7.3
12-3-18	1180	49.0	41.5	14.8	14-3-18	1180	27.6	23.4	11.9	16-3-18	1179	8.9	7.5	6.8
13-3-18	1400	46.2	33.0	15.3	15-3-18	1400	30.7	21.9	11.7	17-3-18	1400	6.7	4.9	9.1
14-3-18	1400	46.2	33.0	15.1	16-3-18	1399	19.2	13.7	11.3	18-3-18	1400	4.3	3.1	8.5
15-3-18	1380	37.9	27.5	14.8	17-3-18	1379	11.0	8.0	11.2	19-3-18	1379	3.2	2.3	7.4
16-3-18	1340	32.7	24.4	14.6	18-3-18	1340	8.4	6.3	12.4	20-3-18	1338	2.2	1.6	8.8
17-3-18	1400	27.2	19.4	15.0	19-3-18	1400	11.0	7.9	13.8	21-3-18	1400	2.3	1.6	10.3
18-3-18	1300	23.5	18.1	15.0	20-3-18	1300	6.9	5.3	12.3	22-3-18	1300	2.7	2.1	6.6
19-3-18	1250	15.3	12.3	15.7	21-3-18	1248	3.3	2.6	14.6	23-3-18	1248	0.9	3.2	...
20-3-18	400	2.2	5.4	13.8	22-3-18	400	0.8	2.0	...	24-3-18	105	0.8	7.6	...
21-3-18	200	1.5	7.7	...	23-3-18	98	0.4	4.2	...	25-3-18	62	0.1	2.3	...

Note. The opium in every case was collected in two samples, each of which was analysed for morphine content. The figure given in the table is the average of these.

The duplicate analyses agreed very closely in every case.

The curves in Chart VI show the maximum and minimum temperatures and also the daily yield per 1,000 capsules and the morphine content of opium from the first, second and third lancements.

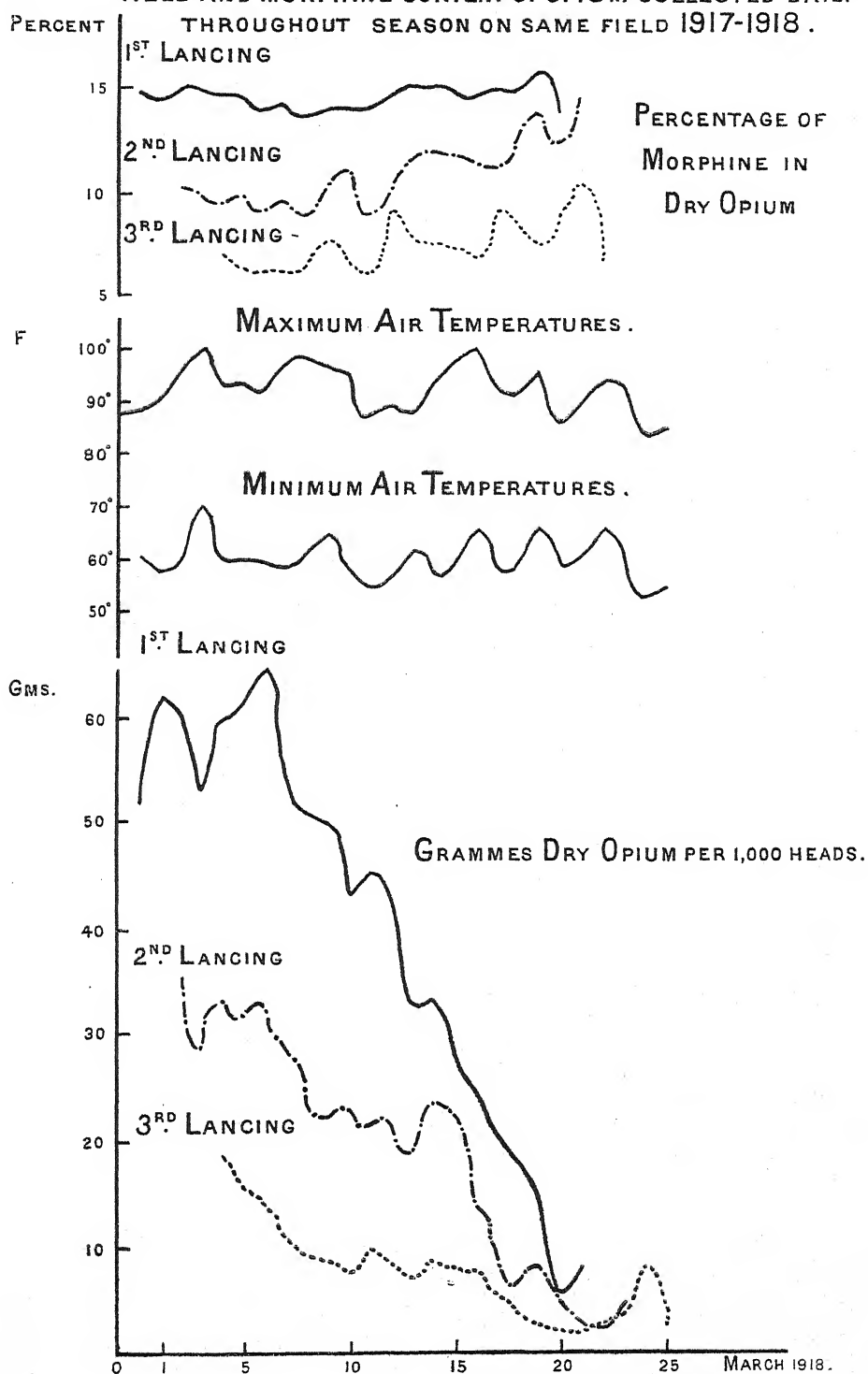
Taking first the morphine content of the opium from the first lancing, it will be seen that, over a period of 21 successive days, there has been practically no variation. Certainly the variation is well within the experimental error which for a morphine content of 15 per cent. has been found to be about  $2\frac{1}{2}$  per cent. This is very interesting in view of the fact that the yield of opium per 1,000 capsules (*vide* Chart VI) showed a rapid falling-off after the seventh day.\* The morphine content of the opium of the second and third lancements does not show such a constant composition. There is considerable day-to-day variation, and, on the whole, a tendency to rise as the season advances. It would seem as though the morphine content of the second and third lancements begins to increase when the yield of opium of the first lancements begins to fall off. That is to say, the morphine content of the second and subsequent lancements depends to a certain extent on the amount of opium yielded in the first lancing. As regards morphine content the nature of the curve indicates no connection between it and weather conditions. The writer would draw attention here in passing to the striking fall in morphine content of the opium from each successive lancing.<sup>1</sup>

The yield of opium might reasonably be expected to show some relation to weather conditions. In fact, it is well known that in cloudy weather or during east winds the yield falls off. After the 7th March the yield fell off so rapidly daily that any difference due to weather conditions would have been quite masked. On the 3rd March, however, the maximum and minimum temperatures showed a big rise, and on that day a distinct fall was observed in the yield of opium per 1,000 capsules, both at the first and second lancements. On that day there was no collection from the third lancements. A study of the curve for the yield of opium at the first lancing would indicate that it was the reciprocal of the temperature curves up to the 9th February: it seems strongly indicated that there is a connection between yield of latex and temperature conditions, the yield decreasing with rise of temperature, and increasing with a fall of temperature. The falling-off in yield after the 7th March is probably due to the drying-up of the fields and the crop. In order to continue

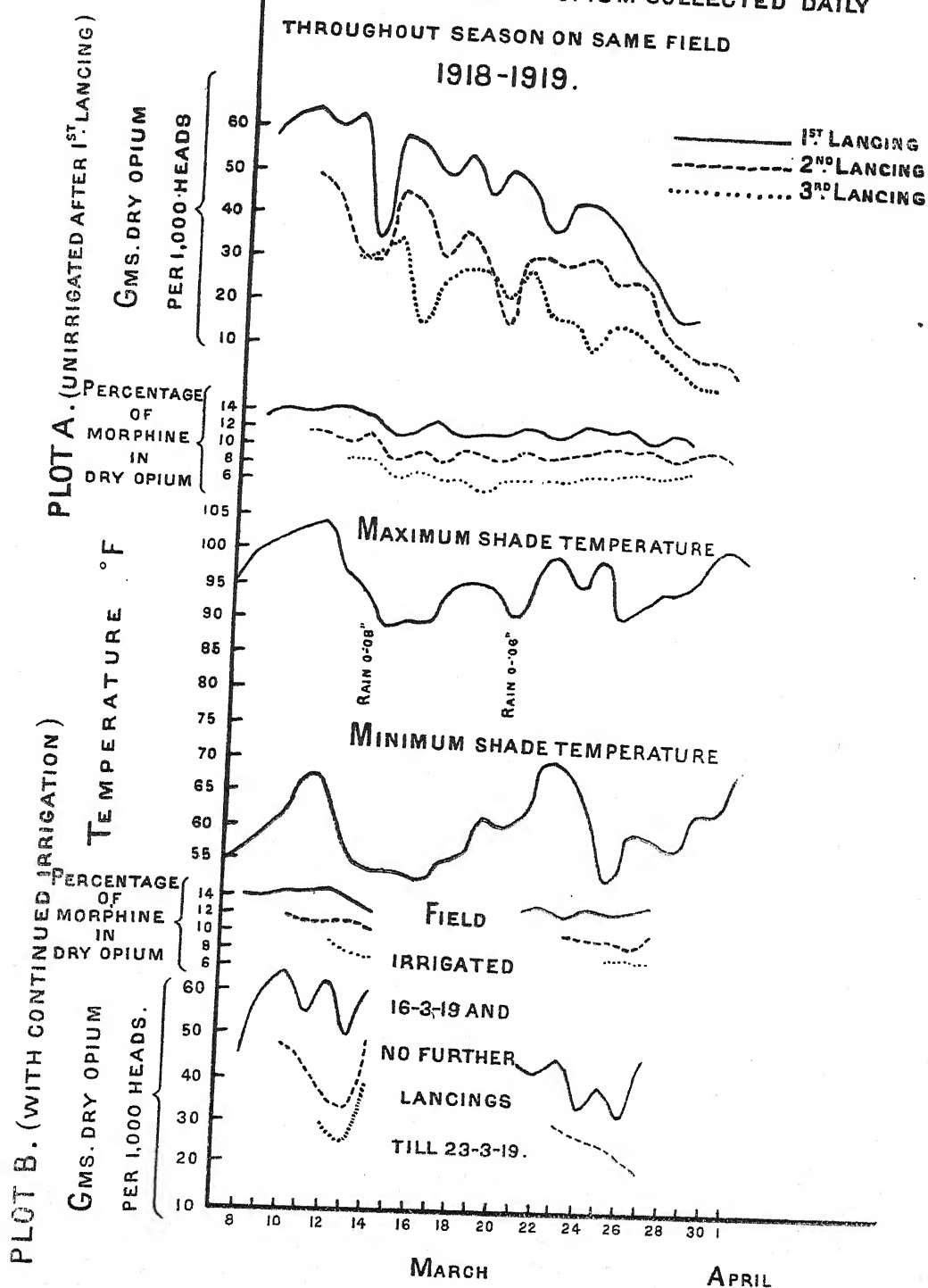
\* In this connection it is also of great interest to recall the fact that though the yield per capsule may be doubled by the use of nitrate as a manure, yet there is no appreciable effect in the morphine content (*see* Influence of Manures, p. 89).

<sup>1</sup> See also Annett, Sen and Singh. *Mem. Dept. Agri. India, Chem. Ser.*, vol. VI, no 1.

YIELD AND MORPHINE CONTENT OF OPIUM COLLECTED DAILY  
THROUGHOUT SEASON ON SAME FIELD 1917-1918.



YIELD AND MORPHINE CONTENT OF OPIUM COLLECTED DAILY  
THROUGHOUT SEASON ON SAME FIELD  
1918-1919.



the lancing daily, irrigation had to be discontinued after the first lancing. In the season 1918-19 means were devised of continuing irrigation throughout a similar experiment.

### 1918-19 *Experiments.*

This year's experiment was similar in general plan to that carried out in 1917-18 with the exception that the field was divided into two portions A and B. Portion A received no irrigation after lancing commenced, whereas portion B received irrigation throughout the experiment in order to keep the land just moist. The number of capsules available for daily lancing on each portion was not always sufficient to provide duplicate samples of opium, but the collection of duplicate samples was aimed at as far as possible. The analyses of these usually agreed, and the occasional differences were not of an important nature. The average figures only are inserted in the following tables. The results are set out graphically in Chart VII.

*Morphine content and yield of opium per 1,000 capsules recorded daily throughout duration of opium harvest, 1918-19.*

Plot A { No irrigation after first lancing.  
500 capsules lanced at each lancing.

FIRST LANCING			SECOND LANCING			THIRD LANCING		
Date of lancing	Grm. dry opium per 1,000 capsules	Morphine per cent. on dry opium	Date of lancing	Grm. dry opium per 1,000 capsules	Morphine per cent. on dry opium	Date of lancing	Grm. dry opium per 1,000 capsules	Morphine per cent. on dry opium
9-3-19	58.2	13.3	11-3-19	49.3	11.6	13-3-19	31.4	8.6
10-3-19	63.4	14.2	12-3-19	44.4	11.0	14-3-19	29.4	8.6
11-3-19	64.2	14.1	13-3-19	32.2	10.6	15-3-19	34.3	6.9
12-3-19	61.0	14.2	14-3-19	28.9	11.4	16-3-19	15.2	7.2
13-3-19	63.6	14.3	15-3-19	46.5	8.5	17-3-19	24.4	6.5
14-3-19	34.9	13.7	16-3-19	43.0	9.2	18-3-19	27.7	6.6
15-3-19	58.5	11.7	17-3-19	31.5	8.2	19-3-19	28.4	5.1
16-3-19	56.3	11.8	18-3-19	36.8	9.6	20-3-19	21.6	6.5
17-3-19	49.8	13.1	19-3-19	30.1	9.4	21-3-19	28.1	6.9
18-3-19	54.8	11.4	20-3-19	15.8	8.6	22-3-19	18.5	6.7
19-3-19	46.0	11.7	21-3-19	31.0	10.1	23-3-19	17.3	6.3
20-3-19	51.8	11.5	22-3-19	31.1	9.1	24-3-19	10.4	7.1
21-3-19	48.0	12.8	23-3-19	30.4	9.6	25-3-19	16.2	7.1
22-3-19	37.2	12.2	24-3-19	32.0	9.9	26-3-19	15.0	7.5
23-3-19	43.5	11.8	25-3-19	27.1	10.5	27-3-19	11.0	7.5
24-3-19	44.0	13.2	26-3-19	27.2	10.1	28-3-19	7.0	7.2
25-3-19	40.2	12.5	27-3-19	21.8	11.1	29-3-19	8.8	8.2
26-3-19	33.0	12.8	28-3-19	12.4	9.5	30-3-19	2.6	..
27-3-19	26.2	11.4	29-3-19	8.4	10.0	31-3-19	1.85	..
28-3-19	18.5	12.4	30-3-19	9.4	10.6	1-4-19	0.64	..
29-3-19	19.3	11.6	31-3-19	5.6	9.4	2-4-19	1.04	..



Plot B. { with irrigation.  
500 capsules lanced at each lancing.

FIRST LANCING			SECOND LANCING			THIRD LANCING		
Date of lancing	Grm. dry opium per 1,000 capsules	Mor-phine per cent. on dry opium	Date of lancing	Grm. dry opium per 1,000 capsules	Mor-phine per cent. on dry opium	Date of lancing	Grm. dry opium per 1,000 capsules	Mor-phine per cent. on dry opium
9-3-19	45.4	14.3	11-3-19	48.3	12.0	13-3-19	28.7	8.9
10-3-19	60.3	14.2	12-3-19	43.2	11.3	14-3-19	25.2	7.6
11-3-19	64.8	14.6	13-3-19	35.4	11.2	15-3-19	38.1	6.9
12-3-19	55.2	14.6	14-3-19	33.0	11.5	....	..	..
13-3-19	62.3	14.9	15-3-19	48.6	10.2	....	..	..
14-3-19	50.2	13.7						
15-3-19	60.1	12.1						
22-3-19	44.7	12.6	24-3-19	30.0	9.8	26-3-19	14.0	7.1
23-3-19	41.5	13.4	25-3-19	27.6	9.7	27-3-19	10.2	7.3
24-3-19	45.2	12.0	26-3-19	26.3	9.4	28-3-19	12.0	6.8
25-3-19	33.8	13.3	27-3-19	22.4	8.7			
26-3-19	38.6	12.5	28-3-19	18.7	10.1			
27-3-19	32.2	12.9						
28-3-19	44.9	13.4						
1-4-19	25.3	13.0	3-4-19	10.4	11.7			
2-4-19	4.8	11.0						

Plot B was irrigated on 16th March 1919 and again on the 29th March. This fact explains the gaps in the table immediately after those dates, since the men are unable to walk on the fields to lance the capsules for some days after irrigation.

On the whole, the results are very similar to those of the previous year. The yield of opium has shown a steady decline with advance of season, and yet despite this, the morphine content of the opium of the first lancements has remained fairly constant from day to day. There seems no essential difference between the results on field A, which had no irrigation after the first lancing, and on field B, which was kept constantly moist throughout by irrigation. This is rather unexpected, since it was anticipated that in the moist field capsules would continue to give their maximum yield for a longer period.

There seems, however, a quite good instance of effect of temperature on yield of latex. Both in Plots A and B on 14th March there was a sudden drop in yield of latex from the first, second, and third lancements with a strong recovery on the following day. The temperature curves shew that there had just previously been a rapid rise in temperature which reached its maximum of 105°F. on 12th March. On the 15th March there was a sudden recovery in the latex yield in every case, and this may be connected with the sudden return to cooler

weather after the 12th March. If the temperature conditions were responsible for these changes in latex flow, it would seem that the effect is not immediate, thus temperatures of the 12th March did not have their effect until two days later. The writer's work on palms<sup>1</sup> shows that in their case the temperature effect is immediate, *i.e.*, cold nights are associated with increased flows of sap and *vice versa*.

In the course of other experiments several instances have been met with which tend to show that yield of opium is influenced by varying weather conditions. The following is a good example of this. It occurred in 1916-17 at Sitoli during an experiment to test the relative values of lateral and terminal capsules as morphine producers.

On the 2nd May 1917, the fifth lancing from the terminal capsules gave double the yield which was obtained at the fourth lancing. Usually a big falling off in yield is to be expected at the fifth lancing. But on that same day the first, second and third lateral capsules all gave bigger yields for their fourth, third and second lancings, respectively, than were expected. The figures are set out in the following table :—

Date of lancing	TERMINAL CAPSULES		1ST LATERAL CAPSULES		2ND LATERAL CAPSULES		3RD LATERAL CAPSULES	
	No. of lancing	Grm. dry opium per 1,000 capsules	No. of lancing	Grm. dry opium per 1,000 capsules	No. of lancing	Grm. dry opium per 1,000 capsules	No. of lancing	Grm. dry opium per 1,000 capsules
29-4-17 . .	4th	11.61	3rd	9.25	2nd	8.83	1st	4.25
2-5-17 . .	5th	23.35	4th	11.30	3rd	17.38	2nd	13.33

This sudden jump in the yield on May 2 can only be accounted for by some sudden change in the weather conditions. It is unfortunate that no record was kept of these at Sitoli.

#### CONCLUSIONS.

1. Climate does not have an important effect in determining the morphine content of opium. The experiments, from which these conclusions are drawn, were carried out on a large scale and the same pure race of poppy was used at the various centres.

<sup>1</sup> Mem. Dept. Agri. India, Chem. Ser., vol. II, no. 8

2. Varying weather conditions seem to be practically without influence on the morphine content of opium.

The experiments, however, indicate that yield of opium is influenced by weather conditions. High night and day temperatures seem to result in a low yield which is increased, however, during cold weather. Hence, although the percentage of morphine in the dried opium is unaffected by weather conditions, the actual yield of morphine is so affected owing to the variations in yield of opium with different weather conditions.

### THE FUNCTION OF ALKALOIDS IN PLANTS.

Winterstein and Trier<sup>1</sup> give a very good general summary of the theories which have been advanced relating to the functions of alkaloids in plant life.

Henry<sup>2</sup> states that the discussion as to the mode of formation and the function of alkaloids in plants has been mostly speculative and that there are comparatively few experimental data available capable of throwing light on either question. In summarizing the function of alkaloids in plants he states that three views have been held:

- (1) That they are nutritive materials used by the plant in metabolism.
- (2) That they act as protective materials against attack of the plants by animals.
- (3) That they are end-products of metabolism rendered harmless to the plant, and stored for the most part in special cells where they are not readily re-absorbed into the active plant tissues.

He states that the third view is now generally held, though recent work on the subject also affords some support to the view that certain of the alkaloids are plastic materials used in plant metabolism.

Haeckel<sup>3</sup> considered that his work supported the first view that alkaloids are used by the plant in metabolism. Tunmann<sup>4</sup> states this is not true at all events for Strychnos. Weevers holds that Xanthine bases, Molisch that Piperin and Wijsman that Cytisin are reserve substances and Comère has experimentally shown that *Ulothrix subtilis* and *Spirogyra crassa*, in the absence of other sources of nitrogen, can utilize atropine, cocaine, and morphine salts directly.

<sup>1</sup> *Die Alkaloide*, p. 263 et seq.

<sup>2</sup> *Plant Alkaloids*, p. 10.

<sup>3</sup> Ed. Haeckel, Sur. L'utilisation et les transformations de quelques alcaloïdes dans la graine pendant la germination. *Compt. rend.*, 1890, CX, 88.

<sup>4</sup> Tunmann. *Pflanzen microchemie*, p. 263.

Clautriau<sup>1</sup>, however, found that alkaloids could not be used as a source of nitrogen by higher plants. For further references to this matter see Muller.<sup>2</sup> The latter concludes that alkaloids are used for formation of protein in developing seed.

The literature (*cf.* Muller, Kerbosch) shows that poppy seeds contain at most inappreciable quantities of alkaloids, so that it is impossible for the alkaloids to be of use to the germinating seed in the case of the poppy plant.

L'Errera<sup>3</sup> was inclined to support the second view that alkaloids are to be considered as protective substances against animal and vegetable parasites, because of their poisonous properties, and of their occurrence in outside tissues, and in wounded cells (*cinchona*, *atropa*, *corynanthe*) and because they are found in the slime surrounding the cotyledons of *Strychnos*. He notes with interest their occurrence in raphide cells (*Amaryllidaceæ*) which produce poisoned wounds on enemies. This places raphide cells on a par with the stinging hairs of *Urticaceæ*. He admits that exceptions occur, *e.g.*, that morphine can be eaten by pigeons, *Datura fastuosa* by goats, *Strychnos* and *Atropa* fruits by birds, *Atropa* leaves by beetles (*Haltica atropæ*), hares and rabbits.

Bayliss<sup>4</sup> makes the following remarks with reference to alkaloids. "It is remarkable how great a variety of these active substances are formed by plants. It seems evident that they may be more or less accidental products of chemical change. A very small number would suffice for protection of the plant from being consumed by animals for food. Similar conclusions may be drawn from the occurrence of adrenaline and a substance related to digitalis in the paratoid glands of a tropical toad described by Abel. It is impossible to see what use to a toad a rise of blood pressure in the animal which attacks it could be."

He<sup>5</sup> also remarks: "The meaning of the enormous variety of toxic and other alkaloids produced by plants is very difficult of explanation. It would seem that if their presence was merely to avoid being eaten by animals one or two distasteful substances would have sufficed. It may be that they are in many cases, as it were, accidental by-products of metabolism although the possibility of some hitherto unknown action on nutritive processes must not be forgotten."

<sup>1</sup> Clautriau. *Nature et signification des alkaloides vegetaux*, Bruxelles, 1910.

<sup>2</sup> Muller. *Archiv der Pharmazie*, 252, 1914, 4. p. 293.

<sup>3</sup> L'Errera. *Efficacite des structures defensives des plantes*. *Lull Soc. Bot. de Belges*. 1886, XXV, Sep.

<sup>4</sup> Bayliss. *Principles of General Physiology*, p. 727.

<sup>5</sup> *Principles of General Physiology*, p. 360

Winterstein<sup>1</sup> and Trier incline to the third view that the alkaloids are waste end-products of metabolism, which have the advantage of being poisonous to animals which would otherwise consume them.

The writer's studies of the factors influencing morphine content in opium might reasonably be expected to throw some light on the function of alkaloids in plants. Before considering this, assistance will be rendered by the setting out of certain facts which the work has definitely established. These facts are taken from the work recorded in this paper and from the paper<sup>2</sup> written in collaboration with H. D. Sen and Har Dayal Singh on the influence of non-environmental factors on the alkaloidal content and yield of latex by the opium poppy.

1. When a capsule is lanced for the first time the concentration of morphine in the latex is at a maximum in the first latex to flow out. As the flow continues the morphine concentration diminishes.

2. At each successive lancing the morphine content of the opium obtained decreases rapidly and if sufficient successive incisions are made latex can eventually be obtained which contains no morphine as measured by the method of the British Pharmacopœia. The morphine is not replaced in the latex by codeine or narcotine. The intervals of time between each successive lancing whether one or five days have no influence on the rate of fall in morphine content of opium from each successive lancing. If the interval of time between the first, second and third lancing is only a few hours, the fall in morphine content at each successive lancing is not so rapid.

3. If only small incisions are made at each successive lancing, and therefore only a small amount of latex removed each time, the fall in morphine concentration of the latex at each successive lancing is much less rapid than when large incisions are made and more latex removed.

Similarly when for any reason, *e.g.*, bad weather conditions or the making of smaller incisions than usual, the yield of the first lancing is low, then the falling-off in morphine content is not so great at the second and third lancing as it would have been with a larger yield at the first lancing.

4. In very young capsules, say six days old, the latex is less concentrated in morphine. After the capsules have reached the stage at which they feel firm, say 16 days in the writer's experiments, the morphine content of the dry opium is the same, however long the first lancing is delayed after that stage and however much the yield of opium may vary from day to day.

<sup>1</sup> *Die Alkaloide*, p. 265.

<sup>2</sup> *Mem. Dept. Agri. India (Chemical Series)*, vol. VI, no. 1.



5. Manuring with nitrogenous manures largely increases the yield of latex but the percentage of morphine in the latex is not modified. The yield of dry opium for a particular race of poppy is roughly proportional to the weight of the capsule and incidentally of the plant itself. For convenience of reference a summary of the data bearing on this point is herewith inserted.

1916-1917.

Plot No.	Manurial treatment	Weight of plants unmanured plot = 100 (average of duplicates)	Weight of capsules unmanured plot = 100 (average of duplicates)	Weight of opium per plot (average of duplicates)	Per cent. of morphine in opium (average of duplicates)
<i>Field No. 29.</i>					
8	Unmanured . . .	100	100	100	8.6
14	K <sub>2</sub> SO <sub>4</sub> . . .	103	107	97	9.1
7					
16	Superphosphate . . .	139	128	105	9.8
6					
13	NaNO <sub>3</sub> . . .	122	119	140	10.0
5					
15	K <sub>2</sub> SO <sub>4</sub> + super . . .	130	119	105	9.6
4					
10	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	127	117	139	9.6
3					
12	Super + NaNO <sub>3</sub> . . .	159	155	171	9.8
1					
11	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	179	165	151	9.6
2					
9					

*Field No. 9.*

8	Unmanured . . .	100	100	100	9.4
14	K <sub>2</sub> SO <sub>4</sub> . . .	119	125	113	9.6
7					
16	Superphosphate . . .	138	150	124	10.8
6					
13	NaNO <sub>3</sub> . . .	155	153	150	10.8
5					
15	K <sub>2</sub> SO <sub>4</sub> + super . . .	137	134	124	9.7
4					
10	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	173	168	158	10.8
3					
12	Super + NaNO <sub>3</sub> . . .	215	194	169	11.3
1					
11	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . . .	208	191	189	11.2
2					
9					



1917-18.

Plot No.	Manurial treatment	Weight of plants unmanured plot = 100	Weight of capsules unmanured plot = 100	Weight of seed unmanured plot = 100	Weight of opium unmanured plot = 100	Per cent. of morphine in opium
<i>Plot No. 44.</i>						
9	Unmanured . . . .	100	100	100	100	14.1
8	K <sub>2</sub> SO <sub>4</sub> . . . .	115	103	114	103	14.9
7	Superphosphate . .	133	120	124	120	15.8
6	NaNO <sub>3</sub> . . . .	174	164	177	188	15.0
5	Super + K <sub>2</sub> SO <sub>4</sub> . .	122	123	172	99	13.8
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . .	167	153	161	192	13.8
3	Super + NaNO <sub>3</sub> . .	213	186	203	217	14.9
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	218	180	193	254	14.7
1	Cattle dung . . . .	133	120	127	121	14.7
10	Castor cake . . . .	168	140	152	149	14.5

<i>Plot No. 50.</i>						
9	Unmanured . . . .	100	100	100	100*	13.4
8	K <sub>2</sub> SO <sub>4</sub> . . . .	104	105	103	91	12.6
7	Superphosphate . .	107	98	94	67	13.9
6	NaNO <sub>3</sub> . . . .	162	154	141	189	12.5
5	Super + K <sub>2</sub> SO <sub>4</sub> . .	109	98	95	92	14.8
4	K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub> . .	166	157	155	207	13.7
3	Super + NaNO <sub>3</sub> . .	203	175	183	193	14.7
2	Super + K <sub>2</sub> SO <sub>4</sub> + NaNO <sub>3</sub>	197	167	170	241	15.7
1	Cattle dung . . . .	109	108	103	146	14.7
10	Castor cake . . . .	189	170	169	250	16.7

\* Figures in this column are not so reliable, since different methods of lancing were employed and this caused capsules to dry up before giving their full yield of latex.

1919-20.

Plot No.	Manurial treatment	Weight of 1,000 complete plants unmanured = 100	Weight of 1,000 terminal capsules unmanured = 100	Weight of seed from 1,000 terminal capsules unmanured = 100	Weight of opium per 1,000 terminal capsules unmanured = 100		Per cent. of morphine in opium 1st lancing only
					1st. lancing	Total lancing	
6 & 11	Unmanured . . . .	100	100	100	100	100	14.4
1 & 16	80 lb. NaNO <sub>3</sub> . . . .	142	121	124	106	127	15.0
2 & 15	160 lb. „ . . . .	154	134	143	124	142	15.4
5 & 12	320 „ „ . . . .	124	106	122	113	103	15.4
7 & 10	480 „ „ . . . .	143	120	138	144	130	15.6
4 & 13	640 „ „ . . . .	141	118	132	158	142	16.1
8 & 9	1600 „ poppy cake = 530 lb. NaNO <sub>3</sub> . . . .	200	161	169	153	166	16.0
3 & 14	1600 lb. castor cake = 580 lb. NaNO <sub>3</sub> . . . .	230	181	177	146	193	17.3

6. Morphine exists as such in the latex inside the plant.

7. Even though the capsules are lanced to exhaustion of morphine the seed shows no sign of deterioration, *e.g.*, in germinating powers. It is the universal practice in India to lance as long as the capsules yield latex and the writer knows of no evidence that the seed suffers in any way. Mr. Gill, Superintendent of the Kumaun Government Gardens, has moreover carried out careful experiments to compare the yield of opium from plants grown from seed produced from capsules which have not been lanced for several generations, with that from capsules which have been lanced to exhaustion of latex each year. He found no difference. This would not agree with Muller's<sup>1</sup> theory that alkaloids are used for the formation of seed proteids. The seed when ripe contains no alkaloids, whereas the ripe capsules contain considerable quantities even after a year's storage.

8. Climate and weather conditions do not affect the morphine content of the latex to any appreciable degree; they may, however, have an important effect on the yield of latex.

<sup>1</sup> Muller. *Archiv der Pharmazie*, 252, 1914, 4, p. 280.

Starting with the above established facts, it therefore appears *that morphine is stored in the capsule more than in other parts of the plant*. This agrees with the results of Clautriau<sup>1</sup> who found the alkaloids were most concentrated in the epidermis of the capsule and diminished in amount towards the lower part of the plant. That morphine is most concentrated in the latex of the capsule seems the only explanation of facts 1 and 2 above. This would explain why the latex first flowing from the cut surface is richest in morphine. As the flow continues, the latex in parts of the plant below the capsule, where it is poorer in morphine, has to be drawn on. This theory fits in well with fact 3 also, because if one only takes out very small amounts of latex at each lancing it will naturally take longer before the less concentrated latex below the capsule is drawn on. This may be accounted for by supposing that a few hours is not sufficient to exhaust the latex already in the capsule by one incision. Actual observations show that this flow continues for at least sixteen hours from one cut. If then the second cut is applied within a few hours of the first, the latex exuding therefrom will be partly latex which was already in the capsule and therefore richer in morphine and which with one day's interval between the lancements would have all been exuded at the first lanced surface.

The plant loses the power to produce morphine about the stage that its capsules become firm to the touch (*vide* No. 4).

This explains No. 2, *i.e.*, that the morphine content of each successive lancing falls off. The flow of latex appears by No. 5 to be proportionate to the size and the weight of the capsule and incidentally to that of the plants. The effect of nitrogenous manures is to increase the size and weight of the capsules.

Yet the morphine concentration in the latex remains the same. It would therefore appear that the amount of morphine produced depends on the amount of plant tissue produced. Different races of poppy, however, produce latex of different morphine concentration. It may be that plants yielding a large amount of latex may produce a lower morphine content in that latex and *vice versa*. Data are being accumulated to test this point.

It may be that the amount of morphine produced is proportional to growth whatever the race of poppy. The amount of latex vessels may, however, be a factor varying with race, and hence a capsule with few latex vessels, *i.e.*, one which will give a low yield of latex, may produce opium especially rich in morphine.

<sup>1</sup> Tunmann. *Pflanzenmicrochemie*, p. 297. And Clautriau. Rech. microchimiques sur la localisation des alkaloides dans le *Papaver somniferum*. *Ann. Soc. Belge de Mur*, 1889, XII, 67.

It would therefore appear that, during the period of active growth in the plant, morphine is being produced at the same rate as the plant tissue. This fact is against the view of Bayliss,\* already mentioned on page 145 that alkaloids are more or less an accidental product of chemical change. The plant no longer produces morphine when the seed begins to ripen, *i.e.*, when the period of active growth has ceased. On the other hand, the morphine does not seem to diminish (*see* No. 4).

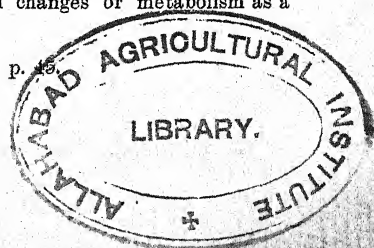
The plant deposits morphine chiefly in the capsule, and one of the functions of the lactiferous system would seem to be the removal of alkaloids to the capsule. This theory is supported by the mode of growth of the lactiferous tubes in the case of *Euphorbia*<sup>1</sup> which send ramifications into each new portion of the tissue in which they occur. No. 7 indicates that the morphine is not used to nourish the seeds. Therefore it would seem that morphine is a useless end-product of metabolism. The animal organism takes in complex food materials, and excretes its end-products of metabolism which are mainly of a simple structure. The plant, on the other hand, feeds on simple substances and therefore it is not surprising if some of its end-products are complex substances, which it finds difficult to excrete. That the amount of morphine produced bears a definite relation to the amount of plant growth, would seem to support the theory that it is an excretory product, for the amount of excretory product would naturally depend on the amount of plant growth.

The fact that the latex of the oldest capsule of a plant is richer in morphine than that of younger capsules (p. 62) supports this view also, since growth is more rapid at the period when the terminal capsules are growing, than when the lateral capsules are growing later. The excretory product morphine would be expected to be produced at a greater rate during active growth, and this would explain why the latex of the terminal capsules is so rich in morphine.

The large number of different alkaloids produced by plants is no objection to the theory that alkaloids are excretory products. Rather it is in favour of it, for certain alkaloids are characteristic of certain families, and the metabolism of different families is certain to show such differences.

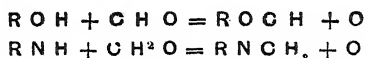
\* *Footnote.* Since the above was written Prof. Bayliss has informed me that his view is not really different from mine. His word "accidental" was meant to imply "useless" in the sense that the alkaloids may be produced by chemical changes or metabolism as a result of other phenomena useful to the plant.

<sup>1</sup> Pfeiffer. *Physiology of Plants*, Trans. by Ewart, vol. III, p. 46.

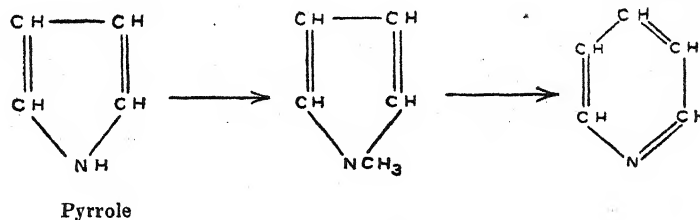


A plant producing much oil in its seed might be expected to form different excretory products from plants producing much starch or proteid as reserve material.

Thus our conclusion closely supports Pictet's<sup>1</sup> views. He has suggested that the secretion of alkaloids represents the plant's means of getting rid of certain products of metabolism. These substances are apparently not toxic to the plants themselves, but have the advantage of being poisonous to animal. Pictet's work is particularly interesting in that it indicates a way in which these alkaloids may possibly be formed in the plant. He suggests that there are two stages in the formation of the alkaloids, the first in which comparatively simple nitrogenous bases are formed during the breakdown of protein, nuclein or chlorophyll. In the second stage these simpler nitrogenous bases form condensation products with other compounds and produce alkaloids. Pictet suggests that methylation of hydroxyl or amino groups by formaldehyde, is one of the commonest changes occurring in plants, *e.g.* :—



The methylated compounds next undergo a molecular re-arrangement and the methyl group enters the ring. Thus a pyridine ring may be produced from methyl pyrrole and he has actually effected this change in the laboratory under the influence of heat.



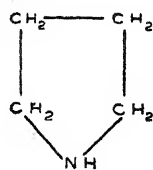
In a similar manner the formation of quinoline and isoquinoline can be explained.

The origin of the pyridine and quinoline rings in alkaloids can therefore be accounted for by assuming them to have been produced from pyrrole or indole rings, which occur in proteins. Pictet isolated a number of simple bases from tobacco, pepper, carrot, parsley and coca leaves by steam

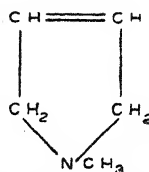
<sup>1</sup> *Archiv. Sci. Phys. Nat.*, 1905. (IV), 1, 9329. *Ber. deut. Chem. Gesellschaft*, 1907, 40, 3771.



distillation in the presence of sodium carbonate, *e.g.*, pyrrolidine and methyl pyrrolidine.



Pyrrolidine



Methyl pyrrolidine

These bodies, by subsequent methylation, become re-arranged and, after condensation with other plant products above, are converted into alkaloids.

Winterstein<sup>1</sup> and Trier suggest that amino acids, *e.g.*, lysine and arginine and carbohydrates, are the most probable starting points for the majority of phytochemical syntheses. They discuss Pictet's results at length.

Ciamician<sup>2</sup> and Ravenna's experiments support those of Winterstein and Trier, since they show that inoculation of plants with pyridine or pyrrolidine derivatives produces scarcely any increase in alkaloidal content, yet similar application of dextrose or asparagine causes a considerable increase.

Robinson,<sup>3</sup> however, makes the following remarks:—"There has been a tendency to explain the results observed by the assumption that plants have at their command enormously powerful re-agents, that are able to cause substances, the properties of which have been investigated with considerable care, to undergo transformations which cannot be induced in the laboratory. To a certain extent and specially in regard to oxidation and reduction, this must be true, but it is probable that this aspect has been exaggerated and that an equally important cause of the variety and complexity of syntheses in plants resides in the highly re-active nature of the substances which function as intermediate products." Robinson is of opinion that the syntheses brought about by plants are frequently simpler than some have supposed. His own excellent work on the synthesis of tropinone<sup>4</sup> describes a very simple method for the synthesis of that alkaloid. This substance is produced by the condensation of succindialdehyde, acetone and methylamine in aqueous solution. He was led to this synthesis simply by an inspection of the structural formula for tropinone. He indicated the method by which alkaloids of various groups may possibly be produced in plants. He has, for instance,

<sup>1</sup> *Die Alkaloide*, 1910, p. 263 *et seq.*

<sup>2</sup> *Year Book of Pharmacy*, 1914, Pres. address, p. 314.

<sup>3</sup> *J. C. S. Trans.*, CXI (11), 1917, p. 877.

<sup>4</sup> *J. C. S. Trans.*, CXI (11), 1917, p. 762.



deduced a scheme for the elaboration of the isoquinoline alkaloids by means of aldo condensations starting from ammonia, formaldehyde, a re-active acetone derivative, and acetyl-glycollaldehyde.

#### CONCLUSION.

Morphine in the opium poppy is a useless end-product of metabolism. The plant having no mechanism for excreting an end-product of such complicated structure stores it in places where it can do no harm to its own metabolism, *i.e.*, chiefly in the capsule. The lactiferous system would seem to represent a means of removing waste products of metabolism.

## PREFACE

THE appearance recently of a paper by B. A. Keen (*J. A. Sc.*, Vol. X, 44, 1920), which gives an admirable résumé of the work so far published on the important subject of soil moisture, has caused me to consider it advisable to give a preliminary account of the lines of work on this subject which have been developed at Lyallpur since 1917. It is probably unnecessary to refer here to the overwhelming insistence of the demand in a Punjab irrigation colony for research into the problems presented by the agriculture of such tracts, which involve the elucidation of the factors controlling the movement of moisture in the soil. That any investigation into these problems involves an attack from many directions is obvious when the complexity of the subject is considered. But to wait for the final convergence of a series of independent lines of investigation unduly postpones the publication of ideas and results which may be of use to workers in the subject and which may interest others who may give assistance. I therefore make no apology for the fact that much of the work described hereinafter is at present very incomplete beyond the statement that I hope at not far distant dates to continue this paper in a series of parts, describing in detail the work which has been performed in each division of the subject.

I wish also to take this opportunity of thanking my assistants by whom the practical part of the work has almost entirely been conducted and in collaboration with whom individually subsequent papers will be published. In particular I wish to refer to Dr. Ramji Narain, who has worked on the absorption experiments; to Bh. Balwant Singh, B.Sc., and L. Parmanand, M.Sc., who have respectively performed most of the work on the compressibility and partition experiments; and last but not least to those whose work has been incidental, or is not yet ready for publication, but who have materially assisted in the progress so far attained, of whom in particular Bh. Jaggat Singh, M.Sc., Assistant Professor of Chemistry, for his work on the vapour pressure measurements, and L. Mukand Lal, L. Ag., for his work on the boring experiments, are to be mentioned.

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# STUDIES IN SOIL MOISTURE, PART I.

BY

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## INTRODUCTORY.

THE importance of our arriving at definite knowledge of the factors which control the movement of soil moisture will be most evident to those acquainted with conditions of irrigation farming in arid tracts. Not only does the productivity of vast areas, as in the Punjab, depend on the proper utilization of the water which irrigation engineering provides for the cultivation of previously desert tracts, but the future fertility of the soil, and its power of supporting an ever-increasing population, are dependent on the methods adopted, for by excessive irrigation, on the one hand, the water-logging of the soil is a not far distant prospect, and an accomplished fact in certain areas, while, on the other, a too parsimonious economy may render the soil saline. Very simple considerations will serve to emphasize these facts.

In the case of the Lower Chenab canal system, which irrigates the tract of country lying between the Chenab and Ravi rivers, records of the level of the sub-soil water table have been maintained by the Punjab Irrigation Department since the canal was constructed. The general form of the water table previous to the construction of the canal, was, as far as can be ascertained from the records existing, saucer-shaped. It is now characterized by marked ridges under the main canal to a distance of about sixty miles from the head works. This is obviously due to direct seepage from the channel and it is in this tract that actual water-logging of the soil has taken place. Below this point there are no marked ridges, but the sections show that there has been a general rise

in the water table. This may be explained by supposing that the canals, which by now are much reduced in size, do not lose so much by seepage, and do not, therefore, produce that marked influence locally which occurs in the higher reaches. It is of great importance, however, to arrive at definite conclusions on this subject. The tendency has been to refer the whole responsibility for the general rise of the water table to seepage from the channels. In fact, Government has seriously considered the feasibility of waterproofing both projected and existing canal systems, but it is obvious that the enormous expenditure which would be involved in such an attempt would be wasted if it were afterwards found that the remedy only partially checked the rise.

Fig. 1, which has been adapted from diagrams published in a paper by C. G. May (*Punjab Engineering Congress Report*, 1918), shows a sketch map of the canal system, and four transverse sections at the points indicated with the water table levels at different years are shown in Fig. 2. In sections *b*, *c*, and *d*, there are no indications of ridges due to seepage from channels such as are exhibited in *a*.

The deepening of the sub-soil stream may be due in part to heading up, caused by the inflow in the area considered being greater than the outflow, and also to the percolation from the surface, which we may suppose will be uniformly distributed over the whole area. Assuming the sub-soil to be uniform, we can obtain an approximate idea of the magnitude of the heading up effect by calculating the gradients round the circumference of an area, and assuming values for the transmission constant of the sub-soil and the depth of the sub-soil stream, the discharges can be calculated. This has been done, but it is found that, even assuming the soil to be composed of coarse sand, the heading up effect does not amount to much more than 1 per cent. of the total rise for a depth of sub-soil stream of 100 feet. We shall, therefore, be justified in referring the rise in the sub-soil at any place almost entirely to the percolation flowing from the surface immediately above it.

By measurement of the contour maps for different years referred to above we can now calculate the volume of water added. In the area cut off by the lines *bb'*—*cc'* this amounts for the 10 years 1905–1916 to  $51.5 \times 10^{10}$  c. ft. and the area *cc'*—*dd'*  $124.1 \times 10^{10}$  c. ft. Assuming the pore space of the soil to be 40 per cent., this increased volume of saturated soil corresponds with quantities of water added of  $20.60 \times 10^{10}$  and  $49.64 \times 10^{10}$  c. ft. To provide this enormous volume of water in 10 years we should require discharges of 653 and 1,574 cusecs respectively. These figures expressed as cusecs per square mile come to 0.65 and 0.59 or, say, 0.62 cusecs. The gross area commanded by the canal is 3,360,000 acres or 5,250 square miles, so the total percolation from

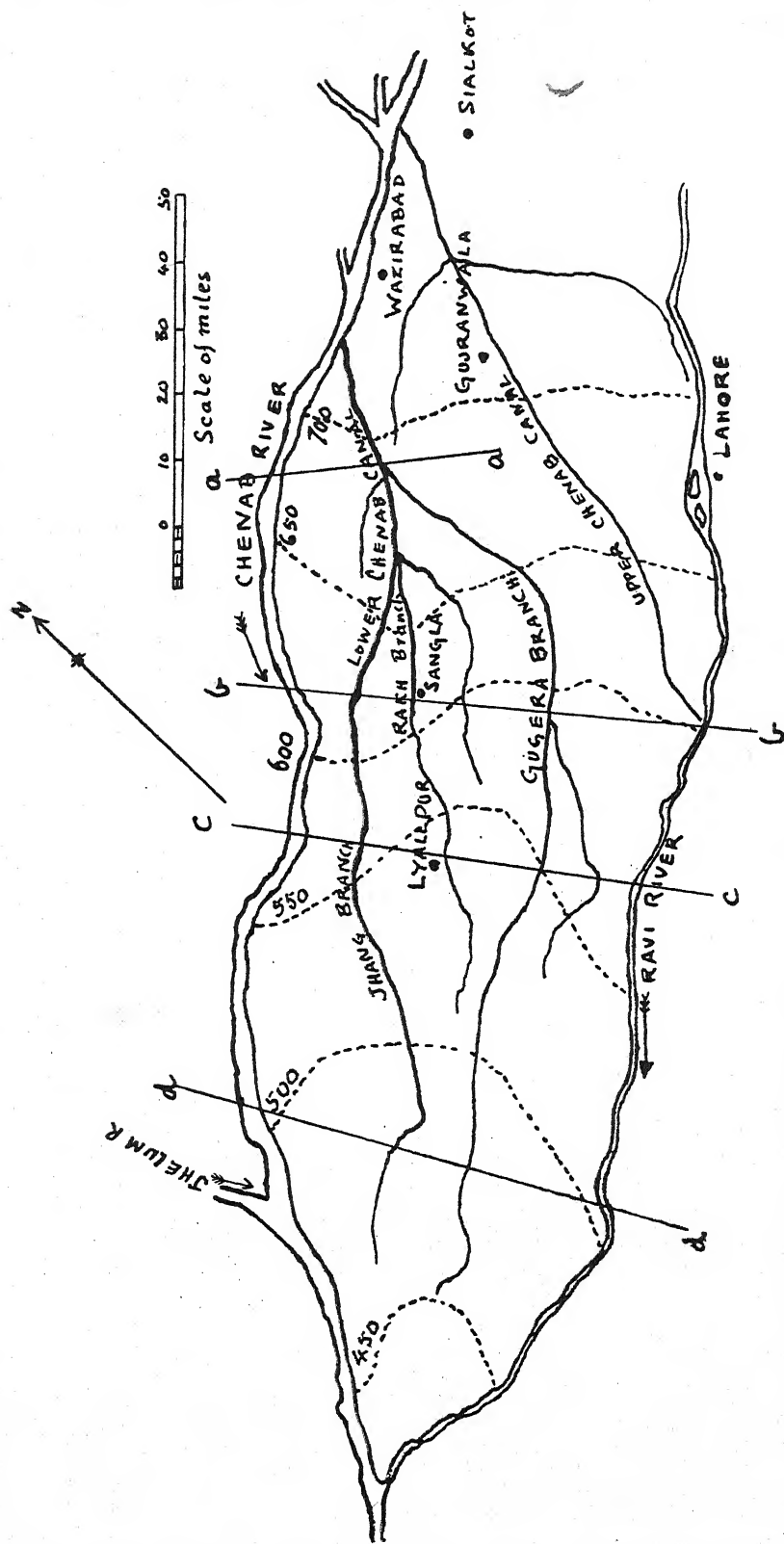


Fig. 1. Upper and Lower Chenab canal systems, showing contours of sub-soil water table at 50 foot intervals and four lines of wells referred to in text.



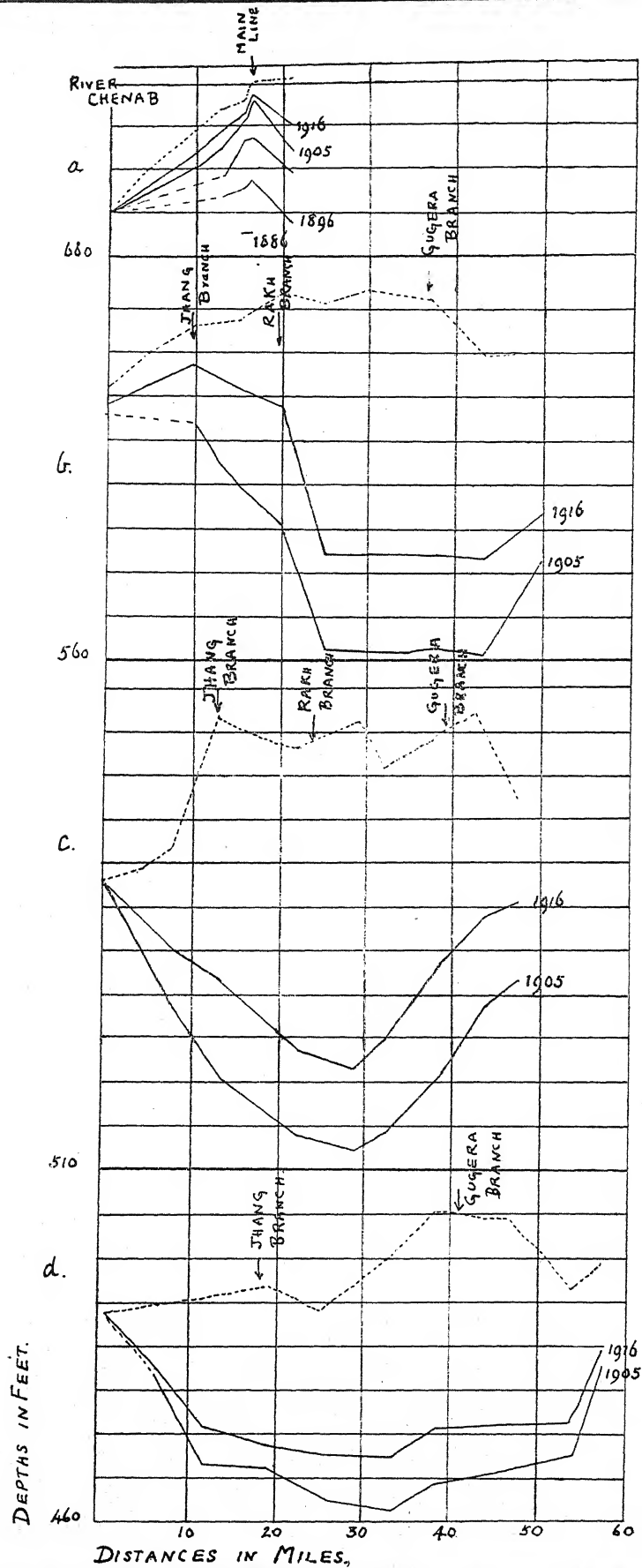


Fig. 2. Cross sections of the Ravi-Chenab Doab, showing water table. The verticle scale is the same throughout, but ,different base lines have been adopted.

the canal system is something in the neighbourhood of 3,255 cusecs and is probably greater than this. The average discharge of the canal may be taken as about 8,500 cusecs throughout the year, so the losses to the sub-soil represent 38 per cent. of the water taken in at the head works. According to the statistics published by the Canal Department, of this, an average of about 17.5 per cent. is lost by absorption in the main line and branches. This leaves about 20 per cent. to be accounted for by loss from distributaries, watercourses and fields, *i.e.*, 1,700 cusecs.

Taking the average irrigated area as 2,250,000 acres, or 3,515 square miles, this loss represents a discharge of about 0.5 cusec per square mile of irrigated area throughout the year.

We have now to try to apportion these losses between the distributaries, watercourses and fields. Kennedy<sup>1</sup> estimated losses from distributaries at 6 per cent. and from watercourses 21 per cent. These figures were obtained by actual measurements of the sinkage of water; it should, therefore, be borne in mind that his figures, representing, as they do, losses due to evaporation as well as percolation, cannot be compared with those based on the actual additions observed in the sub-soil. The losses from the fields he calculated by considering the transpiration ratio of crops, and no great reliance can be placed on this estimate; even if we accept Kennedy's figures for the losses in distributaries, we see that there is a residual loss of about 14 per cent. This loss which takes place in the fields and watercourses will not be affected by the adoption of waterproofing and would appear to be of such a magnitude as to remove even the partial mitigation of the evil of water-logging by this method from the sphere of practical politics.

That there must be a considerable amount of percolation from the fields themselves is obvious when we consider that the irrigation water applied in the field is not pure, but contains a considerable amount of saline material. The average of a large number of analyses made at Lyallpur shows that the total soluble matter in irrigation water is about 12 parts per 100,000, and is almost entirely composed of the sulphate, chloride and carbonate of sodium. We shall therefore be well within the mark in assuming that ten parts of sodium salts per 100,000 of water represents the average value. The total ash of the crops removed from the field contain relatively small amounts of sodium salts, so the only method by which this salt can be removed is by percolation to the sub-soil. The following table gives the amount of irrigation water generally used to mature an average crop, the amounts of sodium

<sup>1</sup> Kennedy. *Pb. Irrign. Branch Papers*, No. 10 (1905), p. 47.

salts added, and the total mineral matter removed from the field with the crop.

Crop	Irrigation in inches	Sodium salts added, in tons	Total ash
Wheat .. .. .	10.0	0.100	0.094
Toria ( <i>Brassica campestris</i> ) .. .. .	10.0	0.100	0.030
Cotton .. .. .	22.5	0.225	0.175

The above crops represent an average three years' rotation, so we may put the average annual gain of sodium salts per acre at between two and three hundredweights.

In most cases the annual application of water will be considerably in excess of what is given above. The content of sodium salts in the sub-soil water of the tract we are considering is between 12 and 24 parts per 100,000. Only in one case has a figure been obtained below 12 and this was for a well near Sangla Hill where there is a considerable out-crop of rocks. In some cases, particularly in a well which has been unworked, the salt content rises high above these figures, but this is probably due to other causes. If we could obtain a reliable average figure for the sodium salt content of the sub-soil water, it should be possible to form an idea of the amount of water percolating to the sub-soil.

To the 2,258,000 acres of irrigated land in the colony between 0.1 and 0.2 tons of sodium salts are added annually per acre, that is about 337,000 tons.

The factor for the conversion of cusecs per year of 365 days to tons of water is 878,400; assuming the sub-soil water to contain 20 parts sodium salts per 100,000 and the irrigation water 10 parts, we have for the total sodium salts percolating to the sub-soil

18 per cent. of 8,500 cusecs containing 10 parts per 100,000

X(20 per cent. of 8,500 " " " " )

337,000 tons from fields.

The concentration will consequently be

$$\frac{8,500 \times 878,400 \times 10}{100 \times 100,000} \left\{ 20X + 18 \right\} + 337,000$$

$$\frac{878,400 \times 3,255}{100,000} =$$

from which  $X = \frac{2}{3}$  nearly

Thus on the above supposition the fields would be responsible for  $\frac{1}{3}$  of the percolation losses taking place after the entry of water into the

distributaries. Before much weight can be attached to such considerations as these, a more detailed investigation, both of the soluble matter contained in sub-soil waters, as also of the salt contents of plant ashes, will be required. Moreover, the determination of the salt concentration of the soil solution will only be of use if it is possible to determine at the same time the concentration of the "free" solution. The salt content of the water percolating from the fields calculated as above works out to only 1 part in 738, which seems much too low, but it must be remembered that the method of calculation does not assume that this figure represents the actual concentration of the solution draining from fields but only the average concentration for the year. Work on this aspect of the problem is being continued.

In the light of these very approximate considerations alone we see that the future of irrigation agriculture can be predicted, and the best practical methods can be devised, only on the basis of a clear understanding of the mechanism of water movement in the soil. It must also be borne in mind that the soluble matter contained in the water will play a large part in determining these actions, and must be considered, not only in the more theoretical aspects of the case, but also in the field. The mere fact that good crops may be grown on one-third the usual amount of irrigation water (*cf.* the work of Howard)<sup>1</sup> is no proof that it is good agriculture until experience has shown that the soil is not impaired thereby, and this may take many years to demonstrate. The experience of the cultivator, with his traditions mainly grounded on "chahi" (well) irrigation, which involves the use of a much more saline water, leads him to put as much water on the soil as he can, and is to be explained by his instinctive fear of his soil becoming saline. Again, we must face the possibility that the adoption of most extensive waterproofing of channels may, at the best, only postpone the day when the introduction of an elaborate soil drainage system will become a necessity for the continued cultivation of the soil. In the meantime, the elucidation of the conditions which determine the movement of water in the soil, will not only enable us to place methods of economy on a scientific basis, but we shall also gain knowledge which will be essential in devising satisfactory remedial measures for the cure of both water-logging and salinity.

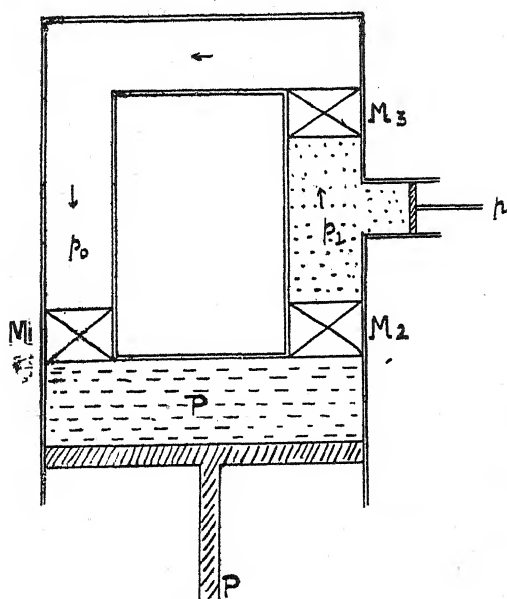
In what follows a theoretical treatment of the factors concerned in the movement of the soil solution is first given.

<sup>1</sup> Howard. *Ag. J. India*, XI (1916), p. 14; and *J. R. S. A.*, LXVIII (1920), p. 555.

(§ 2) EQUILIBRIUM CONDITIONS OF FORCES OPERATIVE IN THE SOIL.

In order to obtain as clear a view as is possible of the conditions of equilibrium of the various forces which will be operative in the soil in determining its behaviour with water, we may best consider a thermodynamic cycle involving the transference of a quantity of water through the various phases in which it exists. This will be done by the method of the osmotic circuit devised by Calendar (*Proc. Roy. Soc., A* Vol. 80 (1908), p. 466), which has the advantage that the precise limits of the integrals developed can be readily seen.

Considering first the case of colloid, pure liquid and vapour phases, we can imagine an arrangement such as is shown in Fig. 3 whereby the circulation of a



quantity of water is maintained at constant temperature by the action of perfectly efficient and reversible engines,  $M_1$ ,  $M_2$  and  $M_3$ .  $M_1$  deals with the work involved in transferring water from the vapour phase, which is maintained at the saturation pressure for the temperature considered, to the colloid which is kept at constant volume by the pressure applied to the piston  $P$ . Similarly,  $M_2$  extracts the water from the colloid and delivers it at the pressure  $p_1$  which is supposed to be determined by the tension of the water films in the soil and represented by the pressure on the piston  $p$ . The

third engine brings the pressure of the vapour to the saturation pressure  $p_0$ . Under these conditions the work performed by the three machines must sum up to zero, and can be written down as follows,

$$\int_P^{p_0} X dp + \int_{p_1}^P W dp + \int_{p_0}^{p_1} V dp = 0 \quad (1)$$

Here  $X$ ,  $W$ , and  $V$  represent the increase in the volume of the phases, to which they refer, which will result from the addition of unit mass of the water at the particular pressure involved. We consequently require to know the

values of  $X$ ,  $W$ , and  $V$  as functions of the pressure. For  $V$ , we may put  $R'T/p$  assuming that the vapour obeys Boyle's Law,  $R'$  representing the ordinary gas constant,  $R=1.98$  calories, divided by the molecular weight of the vapour. Or, since we must consider all the pressures involved in the cycle as including the atmospheric pressure, we should be more accurate to use the values obtained by Regnault for the partial pressures of water vapour in air, assuming of course that Boyle's Law applies and that the partial pressure relation is obeyed. We may thus write, as suggested by Berkeley, Hartley and Burton [*Phil. Trans.*, A 209 (1909), p.177]  $V=p_c/p_o p$ .

For  $W$  we may employ the relation  $W=W_o(1-\alpha p)$  in which we may assume the compressibility  $\alpha$  constant.

For the variation of  $X$  with the pressure we have no certain data, and this will have to be determined experimentally for each soil examined. Ostwald has shown that an expression of the form  $p=KC^n$ , where  $C$  is the volume concentration, and the exponent  $n$  lies between 1.5 and 4.0, agrees with experimental determinations of the swelling pressure  $p$  over wide ranges. By differentiating this expression with respect to  $p$  and  $V$  we get

$$d_v/\sigma'_p = -k' p^n$$

where the exponent is equal to  $n-1/n$  and must consequently lie between 0.33 and 0.75. As will be shown later, this relation can be tested by density determinations of soils suspended in liquids of known osmotic pressure, or, more simply, by density determinations of soils of known water contents in solvents in which water is insoluble.

Substituting these expressions in the integrals, and integrating by parts we obtain

$$mk' (1/p_o^n - 1/p^n) + W_o[(p - 1/2 \alpha p^2) - (p_o - 1/2 \alpha p_o^2)] + R'T \lg p_1/p_o = 0 \quad (2)$$

As an approximate solution of this equation, instead of considering  $X$  and  $W$  as functions of the pressure we may write  $X'$  and  $W'$  to represent the mean values of these factors within the limits of the integrals. We thus get

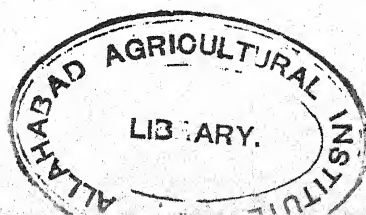
$$X'(p_o - p) + W' (p - p_1) = -RT \lg p_1/p_o \quad (3)$$

or neglecting  $p_o$  which will be small in comparison with  $p$ , we obtain

$$W' p_1 - p(W' - X') = -R'T \lg p_1/p_o \quad (4)$$

We thus see that when the term on the right becomes zero, i.e., when the vapour pressure of the soil is equal to that of the saturated atmosphere,

$$W' p_1 = p (W' - X')$$





This means that if free water is present in the soil its moisture content when in equilibrium with a saturated atmosphere will be determined by the hydrostatic pressure of the liquid films as well as by its colloid content and the specific properties of the colloid. This point is of importance in connexion with the significance which must be attached to the measurement of the Hygroscopic Coefficient of soils, and will be dealt with again after the discussion on film tension. It may also be pointed out that a further term should be introduced for influence of the size of the colloid particles on their swelling pressure. Thus if  $T$  represents the interfacial surface tension between colloid and liquid, the effective swelling pressure will be reduced by an amount which will vary as  $2T/r$ , where  $r$  is the radius of the colloid particles and will be a function of their water content. Actually, since any measurements of the value of  $P$  which it will be possible to make in a soil must also include the effect of variation of the size of the colloid particles, no advantage will result from separating these factors.

We may now introduce the additional complication involved in the fact that we have to deal with a solution instead of a pure solvent. This may be

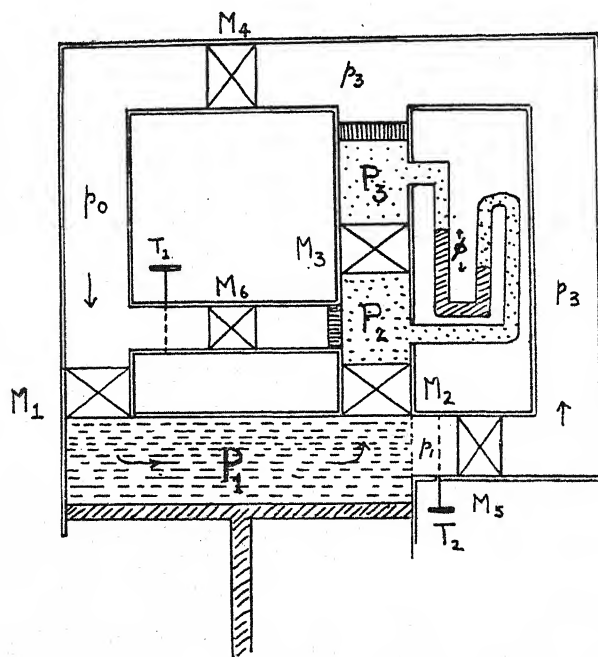


FIG. 4.

done in a similar manner by employing an extra machine to deal with the solution. (Fig. 4.)  $M_2$  now delivers water from the colloid to the solution

at its osmotic pressure  $P_2$ , while  $M_3$  reduces its pressure to the point demanded by the film curvature, and, as before,  $M_4$  brings the vapour to the pressure of the saturated atmosphere. The work terms will consequently be

$$\int_{P_1}^{P_0} X dp + \int_{P_2}^{P_1} V dp + \int_{P_3}^{P_2} V dp + \int_{P_0}^{P_3} V dp = 0 \quad (5)$$

The approximate solution of which will be, neglecting the term involving  $P_0$

$$p(U^1 - X^1) - p_2 U^1 + U^1(p_2 - p_3) = R'T \lg \frac{p_3}{P_0} \quad (6)$$

$p_2 - p_3$  is the same as the hydrostatic pressure due to film tension, and in order to simplify our notation may be written  $\phi$ :  $p_2$  is the osmotic pressure of the solution, measured under the pressure of its own vapour and the partial pressure of the atmosphere, and will in what follows be denoted by  $\pi$ .  $p_1$  is the swelling pressure of the colloid in equilibrium with the solution, and will be denoted by  $\psi$ . Rewriting the equation with these symbols we have

$$\psi(U^1 - X^1) - \pi U^1 + \phi U^1 = R'T \lg \frac{\phi \pi}{P_0} - R'T \lg \frac{1 \pi \phi}{P_0} \quad (7)$$

where the symbol  $\lg \frac{\pi \phi}{P_0}$  refers to the vapour pressure of the liquid of osmotic pressure  $\pi$  under the hydrostatic pressure  $\phi$ . Since, however, it can easily be shown by short circuiting the colloid and placing  $P_0$  in connexion with  $P_2$  through another machine as shown in the diagram, that  $\phi U^1 - \pi U^1 = -R'T \lg \frac{p \pi \phi}{P_0}$  we get the result that for all values of  $\phi$  above zero,  $U^1 - X^1 = 0$

If this is true approximately, and actual measurements of the variation of  $X$  and  $U$  with the pressure will have to be made before the result can be accepted, it means that the moisture content of a soil in equilibrium with an atmosphere of any degree of saturation with water vapour will be determined by the osmotic pressure of the "free" soil solution, and the pressure exerted due to film curvature. This may be made clearer by considering the cycle when the solution is short circuited, *i.e.*, when  $M_2$  is made to deliver at the pressure  $P_3$  in Fig. 4. The work terms will be

$$\int_{P_1}^{P_0} X dp + \int_{P_3}^{P_1} W dp + \int_{P_0}^{P_3} Y dp = 0$$

Whence converting into the new notation and remembering that  $p_3 = \pi - \phi$  we get, from (6) above,

$$\psi(W^1 - X^1) + \phi W^1 - \pi W^1 = R'T \lg p \pi \phi / p_0 = \psi(U^1 - X^1) - \pi U^1 + \phi U^1$$

and,  $(\psi - \pi + \phi)(U^1 - W^1) = 0 \quad (8)$

Thus the swelling pressure of the colloid must be equal to the osmotic pressure, minus the hydrostatic pressure due to film curvature. This equation together with (3) will be the most convenient means of testing the approximate truth of the relations deduced. We may now consider the factors contained in the foregoing equations with a view to finding how far they are susceptible of practical measurement.

(§ 3) THE HYDROSTATIC PRESSURE OF "FREE" WATER  
IN THE SOIL.

As is well known, the work of Briggs<sup>1</sup> resulted in a very general acceptance of the hypothesis which views the soil as a collection of crystalline particles at the points of contact of which "free" water is held by the surface tension of the liquid. Lately, however, the extension of our knowledge of the properties of colloids, and the demonstration of the importance of these properties in explaining absorption phenomena in soils, has necessitated a modification of this originally simple view. It may be pointed out, however, that if any "free" water exists in the soil, the hypothesis of Briggs may be pursued to its ultimate conclusions. The applicability of the relations deduced will obviously depend on the strictness with which experimental facts justify the assumption which regards it possible to consider a soil composed of particles of widely varying sizes and shapes mixed in varying proportions as statistically built up of a collection of equal sized spheres. The work of Slichter and King,<sup>2</sup> and the more recent work of Green and Ampt,<sup>3</sup> shows that such assumptions are fairly well justified as regards the average diameter of the particle and the average cross section and length of the pore spaces. It does not seem unreasonable therefore to suppose that a similar assumption will be justified as regards the average diameter of the soil particles and the number of contacts per sphere. Keen<sup>4</sup> has used the relations deduced by Slichter to calculate the probable capillary rise in soils of various degrees of coarseness, on the assumption that the soil may be regarded as a collection of fine capillary tubes, but himself points out that his treatment does not explain the fact that the moisture content of the soil varies with the height above the free water surface. Actually, we have not to deal with a height in a column of soil throughout which the soil is completely saturated with water, but with a gradually decreasing

<sup>1</sup> Briggs. *U. S. Bureau of Soils Bulletin* 10.

<sup>2</sup> Slichter and King. *19th Rep. (1899), U. S. Geol. Survey*, Pt. II.

<sup>3</sup> Green and Ampt. *J.A.S.*, IV (1911-12), p. I and V (1912-13), p. 1.

<sup>4</sup> Keen. *J.A.S.*, IX (1918-19), p. 396.

concentration. Moreover, it is obvious that it is the distance above the point at which a soil is *saturated* with water which is of importance in practical agricultural considerations. Thus, according to Keen, in a soil composed of particles with diameters ranging from 0.01 to 0.002 mm., the point at which water would completely fill the interstices of the soil, *i.e.*, the point up to which the soil would be saturated, would be 150 feet above the level of water in a well. This estimate although admittedly to be regarded as a maximum value is so much at variance with experience both in the field and the laboratory, that, despite a consideration of the factors which would tend to reduce the height, such as irregularity in the shape of soil particles, the trapping of air in the soil interstices, and the closing of pores by the swelling of soil colloids, it is probable that a more reliable estimate might be formed by considering the problem from another point of view.

If we consider the soil to be composed of equal sized particles, we may suppose the volume of the water held at the points of contact to be that generated by the revolution of the meniscus about the point of contact. We shall probably be sufficiently accurate if we take, as a first approximation, the form of the meniscus to be the arc of a circle which touches the two spheres. We may thus express the volume of water held at the point of contact as a function of the angle,  $2\theta$ , subtended at the centre of the soil particle by the radii drawn from the point of contact of the spheres and the tangent to the meniscus. This relationship gives for  $V$ , the volume of water per contact, the expression (see Appendix)

$$V = \frac{8\pi a^3 \sin^4 \theta}{\cos^2 2\theta} [1 - \tan 2\theta (\pi/2 - 2\theta)] \quad (1)$$

where  $a$  is the radius of the soil particle.

$$\text{The film tension, } \phi, \text{ at a curved surface will be, } \phi = 2\gamma \left( \frac{1}{r_1} - \frac{1}{r_2} \right) \quad (2)$$

Expressing  $r_1$  and  $r_2$  in terms of  $\theta$  we have,

$$r_1 = \frac{a(1 - \cos 2\theta)}{\cos 2\theta} \quad (3)$$

$$\text{and } r_2 = \frac{a}{\cos 2\theta} [\sin 2\theta + \cos 2\theta - 1] \quad (4)$$

$$\text{Consequently, } \phi = \frac{2\gamma \cos^2 \theta}{a} \frac{\sin 2\theta + 2 \cos 2\theta - 2}{(1 - \cos 2\theta)(\sin 2\theta + \cos 2\theta - 1)} \quad (5)$$

When  $1/r_1 = 1/r_2$ ,  $\phi$  vanishes: this means that the pressure immediately inside the film, and outside, will be the same. Neglecting the influence of

gravity, the point at which this takes place should represent the moisture holding capacity of the soil for free water. Thus when  $1/r_1=1/r_2$ ,

$$\frac{\cos 2\theta}{a} [\sin 2\theta + 2 \cos 2\theta - 2] = 0 \quad (6)$$

This equation when solved gives the values  $\theta=0$ , or  $\tan\theta=1/2$ , whence  $\theta=26^\circ 33'$  nearly.

From the values of  $V$  it will now be convenient to calculate the moisture as a percentage on the weight of soil. For this we must know the number of contacts made by each soil particle, and this number will vary with the degree of packing. The number of particles per 100 grams of soil will be given by  $100 \div 4/3\pi a^3 \rho = 31.25/\pi a^3$  taking  $\rho$  the density of the soil as 2.4. If  $c$  = the number of contacts per sphere, then, the number of contacts per 100 grams of soil will be  $n = 31.25/\pi a^3 \times \frac{c}{2}$  since each contact comes in twice. Then, since  $M = n V \sigma$ , where  $\sigma$  = the density of the liquid, which, for the present purposes, may be taken as unity,

$$M = 125c \frac{\sin^4 \theta}{\cos^2 2\theta} [1 - \tan 2\theta (\pi/2 - 2\theta)] \quad (7)$$

Thus we see that for a given moisture content  $\theta$  is independent of the size of the soil particles. Consequently all soils which are packed to the same extent should have the same moisture holding capacity for "free" water. Thus taking the value of  $c$  as 12 (*i.e.*, trigonal packing) and substituting the value of  $\theta = 26^\circ 33'$ , found in equation (6) above, we obtain the value  $M = 23.46$ . It is remarkable that this figure should be approximately the same as that which occurs in the empirical relationship shown by Briggs and Shantz to connect the Moisture Holding Capacity and the Hygroscopic Coefficient of soils, *i.e.*, (Moisture Holding Capacity) = (Hygroscopic Coefficient)  $\times 4.2 - 21$ . Between different soils of the same moisture content and the same packing, the film tension will vary inversely as the radii. Again, if we suppose that the "constants" defined by American investigators as the Hygroscopic Coefficient, the Wilting Point, the Mechanical Equivalent, *et cet.*, represent concentrations of moisture for which the film tension has definite values, and, if we assume the osmotic concentration of the free water the same for all concentrations of water, it is easy to see that a given ratio in the soil is independent of the size of the particles

$$\frac{p}{p_1} = \frac{\phi}{\phi_1} = \frac{f(\theta)}{f(\theta)}$$

Therefore the ratios between the moisture contents of soils under conditions adopted to define the soil "constants" should be independent of the



mechanical constitution of the soil as has been shown by Briggs and Shantz.<sup>1</sup>

It must be remembered that these considerations neglect entirely for the present the influence of the colloids of the soil in determining the "bound" water or the size of the particles. An attempt will be made to introduce this factor later; the conclusions drawn in this section will only hold for "free" water, (not solution) in soils, the sizes of whose constituent particles are independent of the moisture content. An objection has been raised by Trouton<sup>2</sup> to the employment of considerations based on surface tension when the radius of curvature of the film approaches molecular dimensions. It does not appear, however, that this will invalidate the relations deduced for the concentration of moisture above a certain limit. Thus with clay particles of radius  $r=0.002$  cm. for a moisture content of free water of 1.64 per cent. ( $\theta=12$ ) the values of  $r_1$  and  $r_2$  are respectively  $1.2 \times 10^5$  cm. and  $9.3 \times 10^5$  cm.

We may now consider the capillary rise in the light of these deductions. For the relationship between the vapour pressure and the hydrostatic pressure to which a liquid is subjected we may use the relation

$$\phi_0 W_0 = R' T \lg p_1/p_2$$

where  $\phi_0$  represents the value of the hydrostatic pressure of the pure liquid, the pressure of the atmosphere being assumed constant, and  $W_0$  is the specific volume of the liquid which may be assumed constant and equal to unity. But, assuming the vapour obeys Boyle's Law, and the hypsometric formula, we have

$$\lg p_1/p_2 = \lg \sigma_1/\sigma_2 = -gh/k$$

where  $\sigma$  represents the density of the vapour and  $h$  is the distance between the points where the pressures are  $p_1$  and  $p_2$ .

$$\text{whence } \phi_0 W_0 = -R' Tgh/k \quad (9)$$

In C.G.S units,  $k = 7.80 \times 10^9$

$$R' = 84.7 \times 10^6 \div 18 \text{ (the mol. weight of water vapour)}$$

$$g = 981, \quad T \text{ (supposed constant)} = 300^\circ$$

Whence, collecting the constants and substituting, we get,

$$\phi_0 = Kh \quad (10)$$

$K = 177.6$  when  $h$  is measured in centimetres and  $5413$  when measured in feet.

<sup>1</sup> Briggs, Shantz. *U. S. Bureau Plant Industry Bull.* 230, 1912.

<sup>2</sup> Trouton. *Proc. Roy. Soc.*, LXXVII A (1905), p. 292.



Table I shows corresponding values of  $\theta$ ,  $M$ ,  $\phi_0$  and  $h_a$ ,  $a$  being supposed equal to unity.

TABLE I.

$\theta$	$M$	$\phi_0$ in atmospheres	$h_a$ (in feet,
5.0	0.07	0.00890	1.623
7.6	0.24	0.00427	0.777
8.0	0.38	0.00316	0.575
10.6	0.87	0.00186	0.339
12.0	1.64	0.00117	0.301
15.0	3.55	0.00062	0.114
17.5	4.97	0.00043	0.069
20.0	9.37	0.00021	0.039
21.0	10.67	0.00017	0.030
22.0	12.83	0.00013	0.023
23.0	14.84	0.00009	0.017
24.0	17.05	0.00006	0.011
25.0	19.41	0.00004	0.006
26.3	23.47	0.00000	0.000

The curve in Fig. 5 shows the log of  $h_a$  plotted against values of  $M$  corresponding with  $\phi_0$  calculated from equation 5.

To determine the height above the water table at which a given moisture percentage can be maintained, find the point on the curve corresponding with the value of  $M$  required from which  $h$  can be calculated for known values of  $a$ . Thus when  $M = 5$  per cent. the height at which this moisture content will be maintained in soils, having various values for the average diameter of particle, is shown in Table II.

TABLE II.

Average diameter in mm.	Height to which soil would remain saturated according to Keen	Height at which water content will be 5 per cent. in feet
1.0	0.3	0.694
0.1	3.2	6.94
0.01	31.8	69.4
0.001	317.5	694.0

There appear to exist no satisfactory data for the testing of these relations. It is unfortunate that in the experiments of Buckingham<sup>1</sup> no record of the physical properties of the soils used is given. However, a rough method of testing the equation is possible by comparing the graphs published by Buckingham for the equilibrium distribution of water in 40 inch columns of soil, with

<sup>1</sup> Buckingham. *U. S. Bureau of Soils Bulletin* 38.

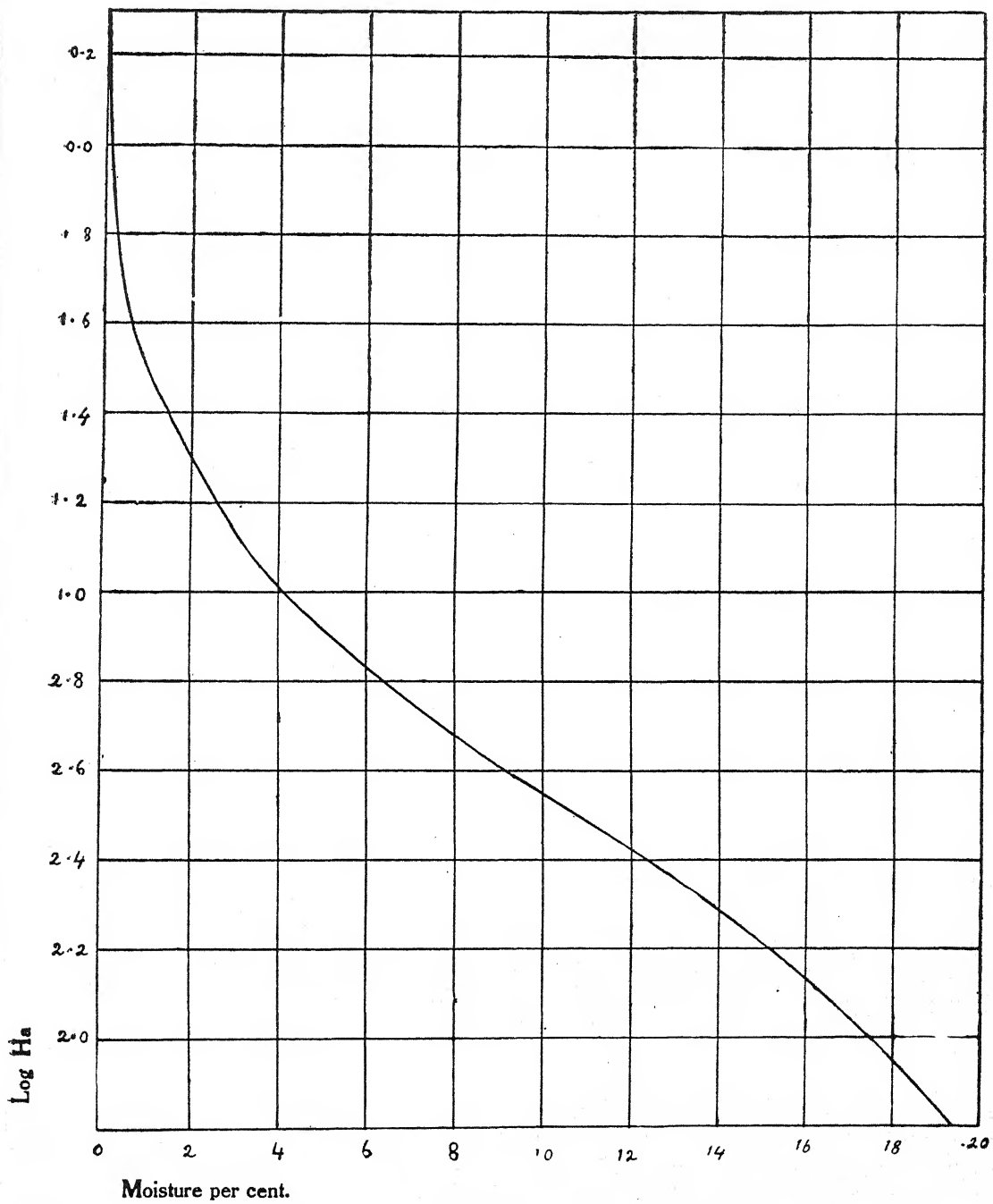


Fig. 5.

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the theoretical curve. Thus in Fig. 12 of the pamphlet referred to, the graphs for three sandy soils are given, namely, New Mexico dune sand, Windsor sand sub-soil and Norfolk sandy loam. Fig. 6 shows the experimental

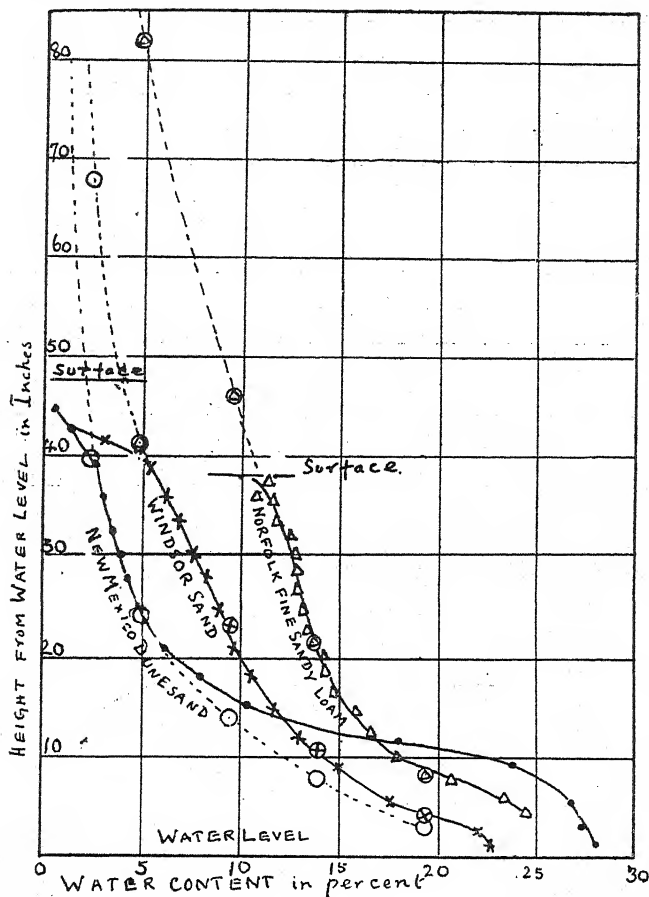


FIG. 6.

Distribution of water in sandy soils after Buckingham, compared with theoretical curves, assuming the following values of  $d$  (the mean diameter) Norfolk sandy loam 0.1 mm., Windsor sand sub-soil 0.2 mm., New Mexico dune sand 0.288 mm.

curve compared with those plotted from the above relations assuming that in all cases the soils were packed to the maximum extent, *i.e.*,  $c=12$ , and that in the case of the three soils the average radii are as shown below:—

Soil	Radius in mm.
New Mexico .. ..	0.345
Windsor .. ..	0.20
Norfolk .. ..	0.10

It is obvious that the expression is of the right order of magnitude ; in fact, the fit of the curves for the two finer soils is reasonably good over the range measured. In the case of the first however at the higher concentrations of moisture the curves appear to be quite different. This may be due to the fact that with the coarser soils divergencies due to the irregular shapes and sizes of the soil particles will be most evident. In any case for the proper testing of these relations a further enquiry into the relationship between the texture of soils and the factor which appears in the above equation as the average diameter will have to be made. For this purpose a method similar to King's aspirator apparatus for the determination of effective diameter suggests itself and has indeed been applied by Green and Ampt. The further discussion of these relations, and their application to the heavier soils, will be continued, after the treatment of the other factors involved, in the ensuing sections.

The application of the considerations given above to the calculation of the " Mechanical Equivalent " which introduces the effect of gravity, which has so far been neglected, is a less simple matter and is left for treatment on a future occasion.

## APPENDIX.

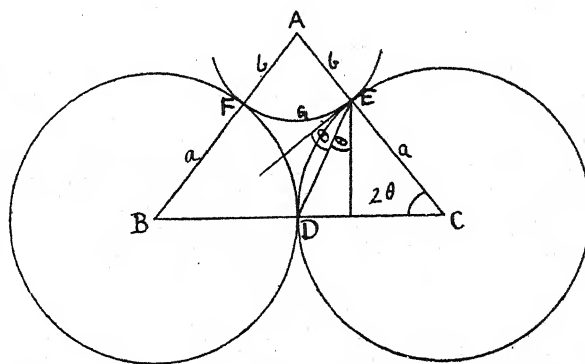


FIG. 7.

$$\text{Area of triangle ABC} = \frac{1}{2} (a + b)^2 \sin 4\theta$$

$$\text{Distance of C.G. from BC} = \frac{1}{3} (a + b) \sin 2\theta$$

$$\text{Area of the two sectors, CED and BDF} = a^2 2\theta$$

$$\text{Distance of their C.G.'s from BC} = \sin \theta \times \frac{2}{3} a \cdot \sin \theta$$

$$\text{Area of sector AFE} = \frac{1}{2} b^2 (\pi - 4\theta)$$

$$\text{Distance of its C.G. from BC} = (a + b) \sin 2\theta - \frac{\sin (\pi/2 - 2\theta)}{(\pi/2 - 2\theta)} \times \frac{3}{8} b$$

If the curvilinear area FGED be  $A$ , and its C.G. from BC be  $\gamma$ , then the volume  $V$  generated by its revolution about D will be  $2\pi\gamma A$ .

$$\therefore V = 2\pi [1/6 \cdot (a+b)^3 \sin 2\theta \cdot \sin 4\theta - 4/3 a^3 \sin^2 \theta - 1/2 (a+b) b^2 \times \sin 2\theta (\pi - 4\theta) + 2/3 b^3 \cos 2\theta]$$

$$= \frac{8\pi a^3 \sin^4 \theta}{\cos^2 2\theta} [1 - \tan 2\theta (\frac{\pi}{2} - 2\theta)]$$

I am indebted to Bhai Balmukhand, M.Sc., for this solution.

#### (§4) THE OSMOTIC PRESSURE OF THE SOIL SOLUTION.

Referring to equation (5) of section 2, we see that it is necessary to evaluate the integrals

$$p_0 \int_{\psi}^{\psi} \psi \times d\psi + \int_{\pi}^{\pi} \psi U d\psi + \int_{\phi}^{\phi} \pi U d\psi + \int_{p_0}^{\phi} \psi W d\psi = 0$$

where the symbols  $\pi\phi\psi$  have been substituted for  $p_1, p_2$  *et cetera*. We can thus determine  $\psi$  if we can measure  $\pi$ , the *true* osmotic pressure of the soil solution and the hydrostatic pressure  $\phi$ . Although the value of  $\phi$  may be small compared with  $\pi$  at concentrations of *free* water above 1 per cent., the pressure increases very rapidly below this point with soils of average fineness, so we cannot afford to neglect this factor until we are able to determine the amount of free water which will be held by the soil under various conditions. If we know the value of  $\gamma$ , the surface tension of the soil solution, we can estimate  $\phi$  for various values of the free water content, on the hypothesis developed in the preceding section. If it were possible to obtain a truly representative sample of the soil solution it would then be possible to determine  $\pi$  directly. The probably insuperable difficulties which would be met in such an attempt are well known, so we are limited practically to the determination of the *apparent* osmotic pressure by physical measurement of the properties of the soil solution *in situ*. This fact has never been clearly recognised. Thus, both the freezing point method adopted by Bouyoucos,<sup>1</sup> and the vapour pressure experiments of Schull,<sup>2</sup> do not take account of the film tension of the free liquid, and in consequence no accurate comparison can be made of the osmotic pressures of the soil solution in different soils, since the film tension will vary inversely as the average radius of the soil particles. Thus referring to the approximate solution given in equation (7) of section 2, we have  $\psi + \phi - \pi = 0$ .

<sup>1</sup> Bouyoucos. *J. A. R.*, VIII (1917), 195, and XV (1918), 331.

<sup>2</sup> Schull. *Bot. Gaz.*, 62 (1916), p. 1.



It is only if we can determine the values of these factors as functions of the free and absorbed moisture content of the soil, that we shall be able to obtain precise knowledge of the conditions under any particular circumstances. Thus we may write provisionally,

$$\phi \propto \frac{\gamma}{a} f' \left( \frac{1}{UM_1} \right) \quad (1)$$

where  $M_1$  represents the free water in the soil.

$$\pi \propto mRT/UM_1 \quad (2)$$

where  $m$  is the number of molecules of dissolved salt in 100 gs. soil.

$$\psi \propto b f'' \left( \frac{1}{XM_2} \right) \quad (3)$$

where  $M_2$  is the adsorbed water,  $b$  is a constant proportional to the colloid content of the soil, and as shown by Ostwald and Mundler,<sup>1</sup>  $n$  is an exponent which will probably lie between 3.5 and 4.0. Then

$$b f'' \left( \frac{1}{XM_2^n} \right) + \frac{\gamma}{a} f' \left( \frac{1}{UM_1} \right) = \frac{mRT}{UM_1} \quad (4)$$

If we can confirm the hypothesis involved in the above expressions, and also assume constant, or determine the variation of  $\alpha$ ,  $b$ ,  $\gamma$ ,  $m$ ,  $U$  and  $X$  with  $M$  and  $T$ , we shall be in a position to deduce an expression which will give a complete account of the water in the soil.

We may first examine equations (1) and (2) to see how far practicable physico-chemical measurements will enable us to confirm the expressions.

Reference has already been made to the freezing point method of determining the apparent osmotic pressure of the soil solution. This, while appearing to be fairly accurate, has the disadvantage that it will only allow us to gain a knowledge of the conditions in soils near the freezing point of water. Schull's method of estimating the vapour pressures in soils has the disadvantage that it is dependent on the physiology of the semi-permeability of the testa of seeds, and is open to the objection that the semi-permeability of a seed may vary with the nature of the soil solution with which the seed is placed in contact. Moreover, since the moisture relations in soils will depend on their degree of packing, it will be advisable to adopt a method in which this variable can be measured. An attempt is consequently being made to determine the actual vapour pressures in soils by purely physical methods. For this purpose, the aspiration method of Walker perfected by Berkeley and Hartley has been selected. It has not yet proved possible to overcome all the practical

<sup>1</sup> Ostwald and Mundler. *Koll. Zeit.*, XIV (1919), p. 7.

difficulties met with in an attempt of this sort, and the results so far obtained are subject to errors which preclude any advantage in the publication of the results obtained.

Besides these absolute measurements, an attempt has also been made to measure the magnitude of the swelling pressure in the soil. In order to check these results a series of experiments has been designed whereby direct comparisons of the moisture equilibrium can be made under varying conditions of moisture content, packing, and salt content. Thus, if two quantities of a soil, packed to the same extent, are separated by a semi-permeable membrane and allowed to come to equilibrium, since the vapour pressures must be equal, from (§2.6) we have

$$UX(\phi_1 - \pi_1) = U_2(\phi_2 - \pi_2) \text{ and}$$

$$\psi_1 X_1 = \psi_2 X_2$$

If then we substitute the tentative expressions for  $\phi$ ,  $\pi$ , and  $\psi$  given in equations (1), (2) and (3) above, we obtain the following relations,

$$U_1 \left[ \frac{2\gamma_1}{a_1} f' \left( \frac{1}{UM_1 - \kappa_1} \right) - \frac{nRT}{M_1 - \kappa} \right] = U_2 \left[ \frac{2\gamma_2}{a_2} f' \left( \frac{1}{U_2 M_2 - \kappa_2} \right) \right] \quad (5)$$

This assumes that the osmotic pressure of the soil to which no salt is added is zero.  $\kappa$  is used to represent the weight of water in the colloid phase, and since the vapour pressures in the two soils must be equal

$$p_1 = p_2, \text{ so } \psi_1 = \psi_2, \text{ and } \kappa_1 = \kappa_2$$

Thus assuming that  $\gamma$  does not vary with the concentration or that we know its variation, and similarly, that  $\alpha$  is known, provided we can determine the variation of the vapour pressure of the soil with water and salt content, we shall have sufficient data to test the equations completely. The assumptions are however so great that it will probably only be possible to use equation (5) in a qualitative way. Firstly, we have no right to assume that we can rely on the value of  $m$ , the number of molecules of salt added per 100 grams of soil, since adsorption may take place, or that we can cancel out the partial osmotic pressure of the salts already in the soil, from the two sides of the equation. Moreover, any semi-permeable membrane which it will be possible to prepare artificially in order to suit the requirements of the experiment will certainly not be semi-permeable with respect to all the dissolved salts in the soil. We shall thus have conditions such as have been discussed by Donnan, and which he terms membrane equilibria. Altogether the system appears so complex as almost to discourage any attempt at the experiment. Moreover, it must not be forgotten that the most perfect semi-permeable "membrane" in nature is the vapour phase. Thus at first sight it would appear that by far the most promising method of attacking the problem would be by allowing soils of different salt

contents to come to equilibrium in atmospheres containing varying amounts of water vapour. Experiments will be described in which this method is adopted and which should enable the equation developed above to be tested under the best conditions, but it may be pointed out, if any justification is needed for the membrane experiments, that the attainment of equilibrium in the soil through the vapour phase is an extremely slow process, and that, in nature, movements of water of any practical importance will undoubtedly take place through the free liquid or colloid phases. Moreover, since the soil itself can at times act as an imperfectly semi-permeable membrane, as observations made in the course of the experiments to be described, and the work of Lynde and Bates,<sup>1</sup> show, and since, as again will be demonstrated experimentally, unequal distribution of soluble matter in the soil, coupled with the functioning of the soil as an imperfect semi-permeable membrane, brings into play osmotic forces which are of particular importance in considering the dynamical aspects of the problem, even if these membrane experiments provide no sure basis for quantitative discussion in the present state of our knowledge, valuable qualitative results may be expected.

It will, therefore, be as well to consider along the lines adopted by Donnan<sup>2</sup> the effect which the partial permeability of the membrane to the more diffusible salts of the soil will have on the results. As has been pointed out above, if any unequal distribution of the soil salts results from membrane action we shall be unable to assume that the total effective osmotic pressure will be given by the term  $mRT/M \cdot \kappa$ . Moreover, there is in addition the error involved in adsorption of salt by the soil colloid. This error we should probably be justified in neglecting as all evidence tends to point to the fact that adsorption of electrolytes by the colloids of the soil is almost invariably attended by the liberation of an equivalent amount of previously adsorbed ions.

Donnan's theory has only been worked out for the simple cases of univalent ions in which either one or no ions are common. In a case such as the equilibrium across a membrane of salts such as calcium ferrocyanide on the one side and sodium chloride on the other, the equations which can be developed by thermodynamical reasoning are of the third order in  $x$  and  $y$ , the amounts of sodium and chlorine ions respectively which diffuse across the membrane, and are consequently too complex to admit of any useful solution. It can be readily seen however that any changes which occur due to the partial permeability of the membrane must result in a diminution of the effective osmotic

<sup>1</sup> Lynde and Bates. *Jour. Phys. Chem.*, XVI (1912), 759.

<sup>2</sup> *Zeitsch. f. Electrochemi*, XVII (1911), 572.

pressure. By comparing the results obtained with an electrolyte such as calcium ferrocyanide on the one hand, and a non-electrolyte like cane sugar, on the other, it will be possible to obtain some evidence of any existence of this selective distribution of soil salts, which would be of the greatest significance in considering the retention by the soil of useful and harmful salts.

#### (§5) EXPERIMENTAL.

As mentioned in the introduction, it is only intended to give in this section a brief description of the methods adopted for the measurement of some of the factors necessary for a more or less qualitative test of the relations deduced in the foregoing sections, and it is hoped to publish a detailed description shortly. The work described below has been confined to the examination of one soil only, the mechanical analysis of which is as follows :—

Soil No. (L2), Square 10, Lyallpur.			
Fine gravel	..	..	.. 0.27
Coarse sand	..	..	.. 5.61
Fine sand	..	..	.. 37.71
Silt	..	..	.. 25.11
Coarse silt	..	..	.. 21.47
Clay	..	..	.. 9.83
TOTAL			.. 100.00
Moisture equivalent	..	..	.. 22.99
Hygroscopic co-efficient	..	..	.. 2.433

#### *The partition experiments.*

Fifty grams of the soil under investigation, a large sample of which had been previously mixed with the required amount of water or solution, were weighed out quickly and placed in a specially constructed steel cylinder provided with a loosely fitting plunger. A pressure previously fixed upon is then applied by means of a hydraulic press, and the discs of soil so formed quickly taken out and placed in airtight jars.

After many failures it has been discovered that a copper ferrocyanide membrane can be deposited on a particular brand of dialysis parchment paper which appears to be perfectly impermeable to calcium and potassium ferrocyanides, and to cane sugar. This was done by forming the paper into a cylinder which was stuck by marine glue on to two pieces of glass tubing of about one and a half inches diameter. One of the glass tubes is closed at one end and into the vessel so formed a strong solution of potassium ferrocyanide is poured and the whole surrounded with a copper sulphate solution. Electrodes were then placed in the inner and outer

vessels and a weak current passed for several hours. It was found that although no great increase in the resistance of the membrane to the passage of the current took place, an even coherent film was deposited which proved quite satisfactory. The paper without the deposition of this film was quite useless, if the moisture in the soil was high, but appears to prevent the movement of salt entering at low concentration of moisture. This is of interest as it points to the fact which has already been confirmed experimentally, that the soil itself at low concentrations of moisture will act as a semi-permeable membrane. The membrane so prepared is then repeatedly washed in pure water, and after cutting into circles of the right diameter kept moistened between folds of filter paper. Two discs of soil, one made up with pure water, and the other with a solution of the strength required to bring the total moisture content of the two discs and the salt content of one, to a predetermined value, are then separated by a membrane and kept in contact by means of glass discs upon which are placed circular brass pieces provided with three hooks which serve to stretch three rubber bands. The whole assembly is then placed in an airtight jar and kept at constant temperature (32°C.) for about four days. At the end of this time the discs are separated and quickly transferred to weighing bottles and weighed. They are then dried to constant weight in a vacuum oven at 100°.

Table III shows a typical series of results obtained with soil B, pressed to ten atmospheres.

TABLE III.

Series			M <sub>1</sub>	M <sub>2</sub>	S	P <sub>1</sub>	P <sub>2</sub>	S <sub>0</sub>	R	c
A	..	..	3.117	6.440	2.557	25.7	53.2	11.1	2.966	39.70
B	..	..	3.282	6.716	2.564	26.1	53.5	20.4	2.046	38.82
B	..	..	3.782	6.712	2.144	29.9	53.1	17.0	1.775	31.94
A	..	..	3.762	6.750	2.134	29.7	53.4	16.9	1.794	31.62
B	..	..	4.153	6.365	1.724	33.9	52.0	14.1	1.533	27.08
A	..	..	4.156	5.670	1.289	37.4	51.0	11.6	1.364	22.73
B	..	..	4.419	6.037	1.294	37.6	51.4	11.0	1.366	21.43
C	..	..	4.810	6.066	1.176	39.9	50.3	9.3	1.261	19.39
C	..	..	5.090	5.870	0.943	42.8	49.3	7.9	1.153	15.06
D	..	..	5.561	6.610	1.140	42.1	50.0	7.9	1.187	15.73
C	..	..	5.252	5.749	0.471	45.8	50.1	4.1	1.095	8.19
A	..	..	5.130	5.290	0.434	47.3	48.7	4.0	1.031	8.20
D	..	..	5.750	6.103	0.493	46.6	49.4	4.0	1.060	8.56
D	..	..	5.655	5.721	0.253	48.6	49.2	2.2	1.016	4.48

In the first three columns, M<sub>1</sub>, M<sub>2</sub> and S represent respectively the weights per 100 gm. of soil of water (M) and salt (S), (M<sub>1</sub>) referring to the disc containing water only, and (M<sub>2</sub>) the disc containing water and



salt. The next three columns give these figures worked out on the basis of water + salt = 100. The last two columns give the ratio of the concentration

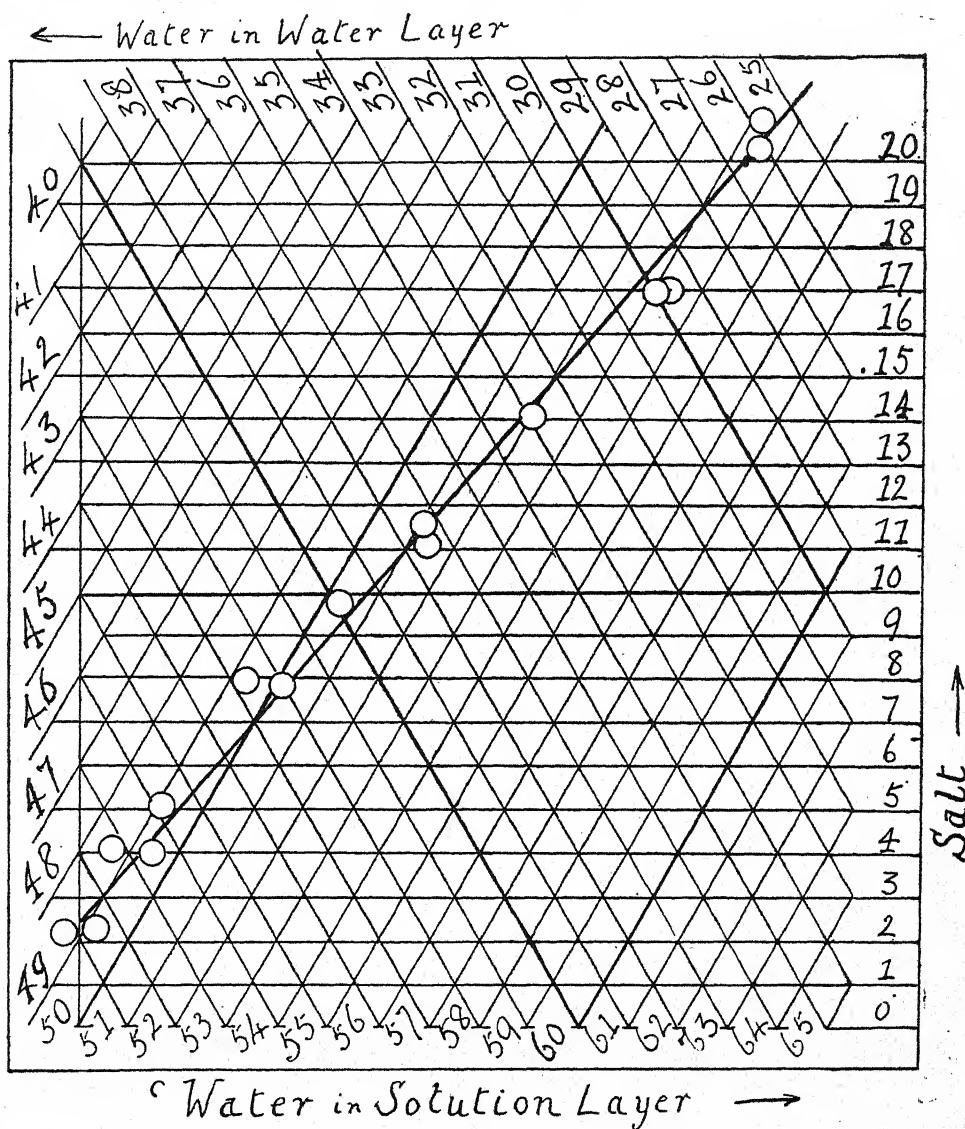
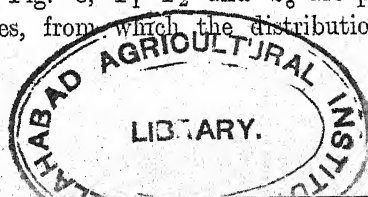


FIG. 8.

Showing partition of water between two soils, one of which contains salt.

of water in the two layers, and the concentration of salt in grams per 100 grams of water. In Fig. 8,  $P_1$ ,  $P_2$  and  $S_0$  are plotted on a system of triangular co-ordinates, from which the distribution of water





and salt in 100 grams of solution can be read off. It will be seen that the distribution follows a straight line law, but that the origin of the curve does not lie at the point where there are equal amounts of water in the two layers. Similar results have been obtained with sucrose solutions, the figures for which will be published subsequently. No explanation can yet be given of this point. A large amount of the error of the observations is probably due to the fact that it is extremely difficult to make up the discs all with the same concentration of moisture: in the experiments recorded above the water content of the discs varied between 5 and 6 per cent. on the total amount of soil. With higher concentrations of moisture at this amount of compression, some water would be expressed from the soil with the higher concentrations of salt.

*The compressibility experiments.*

The compressibility measurements were performed with the same piston and cylinder as was used for making up soils for the partition experiments. A Tangye press working up to a pressure of 90 tons on an  $8\frac{1}{2}$ " ram was used. Two pressure gauges by Schaeffer and Budenburg were used working up to about 180 atmospheres, beyond which point it was not considered safe to go. No means were available for standardizing these gauges, so until this is done, the measurements recorded below must be regarded in the light of preliminary experiments. A diagrammatic representation of the apparatus used is given in Fig. 9. The amount of compression was measured by means of an optical lever as shown in the diagram by means of which a compression of 0.001 cm. could be measured.

The method of procedure was to weigh out a known quantity of soil containing the requisite amount of water or salt solution. This was then placed in the cylinder, the piston inserted, and placed in the press. The mirror was then adjusted to bring the spot on the scale and the pressure increased until the manometer registered the pressure required. Subsequent small increases were made until the pressure remained constant and the spot no longer moved. This usually took about an hour for each reading. The readings so obtained were corrected for the compressibility of the piston. No account was taken of any possible expansion of the cylinder. In any case the correction would be small as the thickness of the cylinder wall was over two and a half inches. The diameter being thus assumed constant the volume of the soil at any point is proportional to the thickness. At a point depending on the percentage of moisture in the soil and also on the amount of salt in solution, the compressed soil becomes saturated, and water flows out from between the



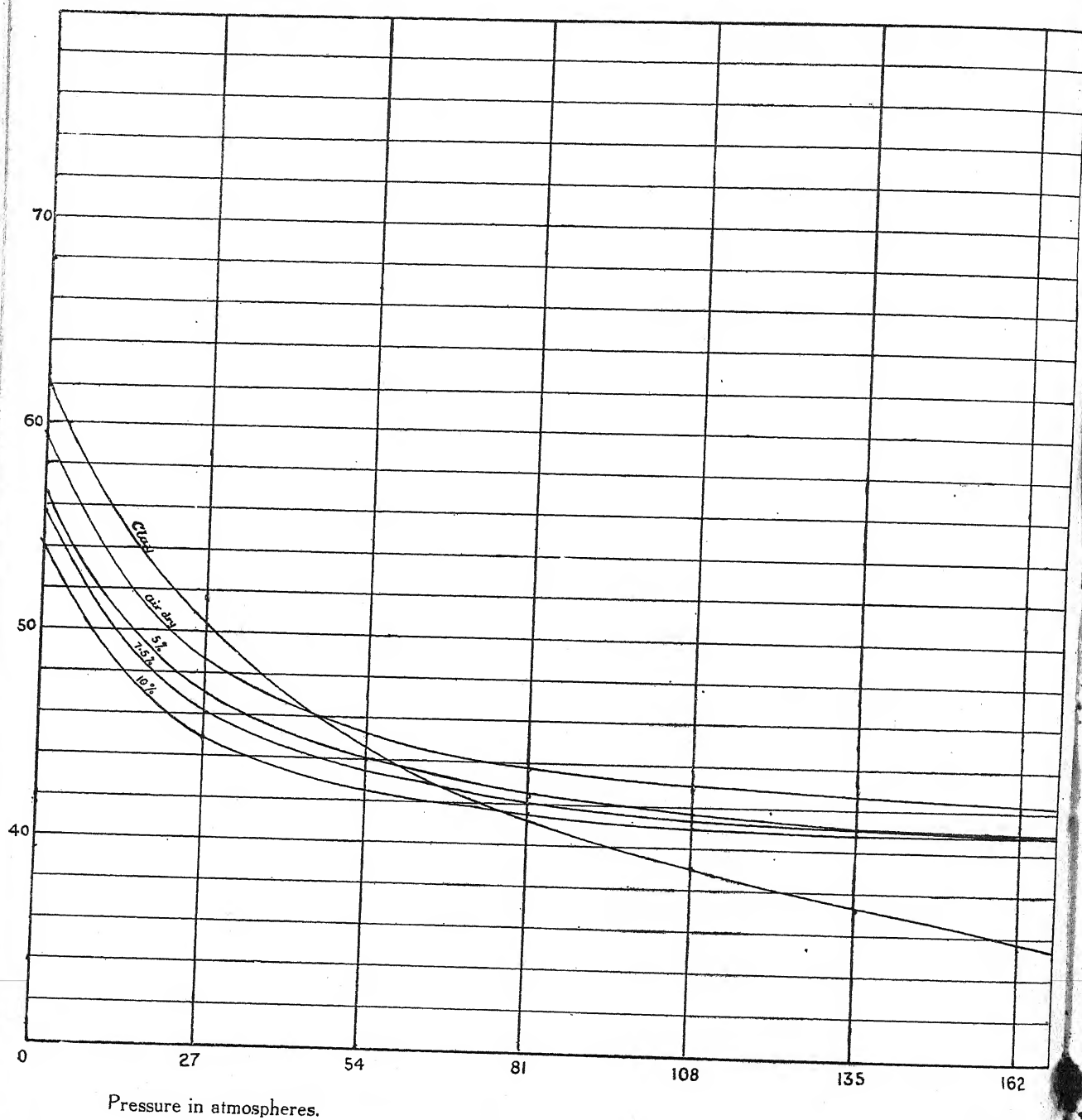


Fig. 10 Showing compressibilities of soil and clay.

cylinder and the bottom plate and into the clearance between cylinder and piston. It will be of interest to determine the exact point at which this occurs,

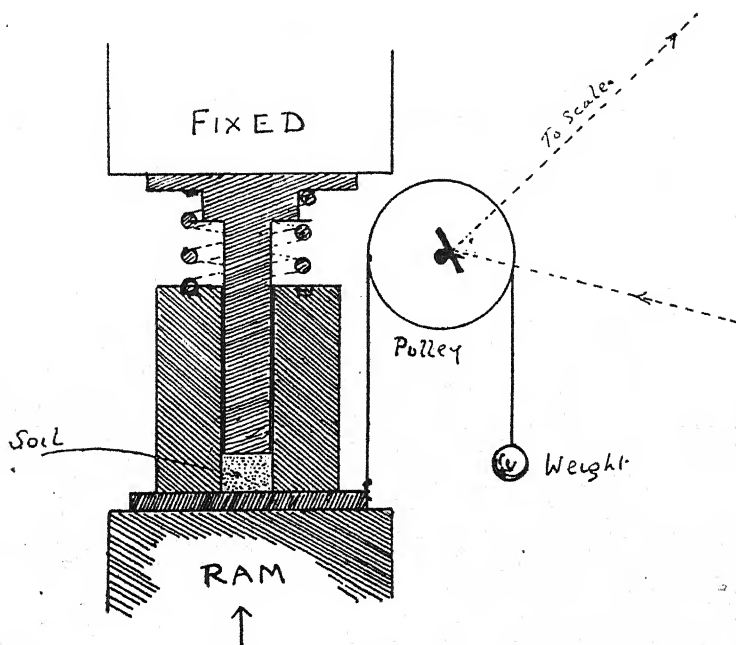


FIG. 9.

but so far no satisfactory method has been devised and only qualitative observations have been possible.

A typical series of smoothed curves is reproduced in Fig. 10. It will be seen that the apparent volume occupied by the soil under any pressure depends on the amount of moisture present, the more water the soil contains, the greater the amount it is compressed. Unless it is possible to determine the amount of pore space at each stage in the compression of the soil, it is obvious that for purposes of estimation of the swelling pressures of the colloid, the experiments will be useless. Moreover, when the pressure is released any free water pressed out will be absorbed again by the colloid. Attempts have been made to measure the reversible changes of volume of the soil with varying moisture contents, but so far have not been successful. Since again the soil will contain free water it will be impossible to determine the pore space by immersion. On this account it was considered advisable to determine the compressibility of "pure" clay isolated from the soil under examination by sedimentation. The curve is shown in the same diagram. It will be seen that

the compressibility of the clay is still very considerable when, with the soil, it has fallen to about the value which would be expected for the crystalline particles contained in it.

*The absorption experiments.*

If a solute can be discovered which is not absorbed by the soil it can be used as a reference substance for the determination of any change in concentration produced by removal of solvent when a soil is allowed to come to equilibrium. Thus on adding soil to a sucrose solution there is a removal of water by the soil colloids which will cause a corresponding increase in the concentration of the sucrose. It should thus be possible to determine the relation between moisture content of the colloid and the osmotic pressure of the soil solution.

The figures given in Table IV and which are plotted in Fig. 11 were obtained by allowing a known weight of soil to attain equilibrium with a known weight of sugar solution of known strength at 32° C.

TABLE IV.

$C_0$ Initial concentra- tion	$C_1$ Final concentra- tion	$x$ Soil gm.	$y$ Sugar sol. in grams	$X$ Gm. water absorbed per gm. of soil
9.383	9.897	20.1429	40.0570	0.102
18.550	18.640	19.9468	53.5341	0.082
18.012	18.660	20.0629	41.9895	0.073
27.080	27.500	20.0810	53.7759	0.047
40.685	41.110	20.0315	45.0864	0.031
43.962	44.340	20.0481	46.3982	0.023
47.876	48.490	20.0412	48.6505	0.033
48.670	49.070	20.0043	60.2483	0.024
56.04	56.360	20.1321	61.8858	0.018

The solution was then removed and the concentration determined by the polarimeter. The columns headed  $C_0$  and  $C_1$  give respectively the initial and final concentrations of sucrose expressed as gm. of sucrose per 100 gm. solution.  $x$  and  $y$  give the weights of soil and solution mixed, from which the values of  $X$  in the last column were calculated. The errors involved in this determination must be very great despite the utmost care taken in the work, as small differences in concentration of strong solutions have to be measured. The results are, however, sufficiently accurate to allow of fair certainty of the course of the curve, but can probably be improved on by the adoption of a more refined method of estimation of the cane sugar. When

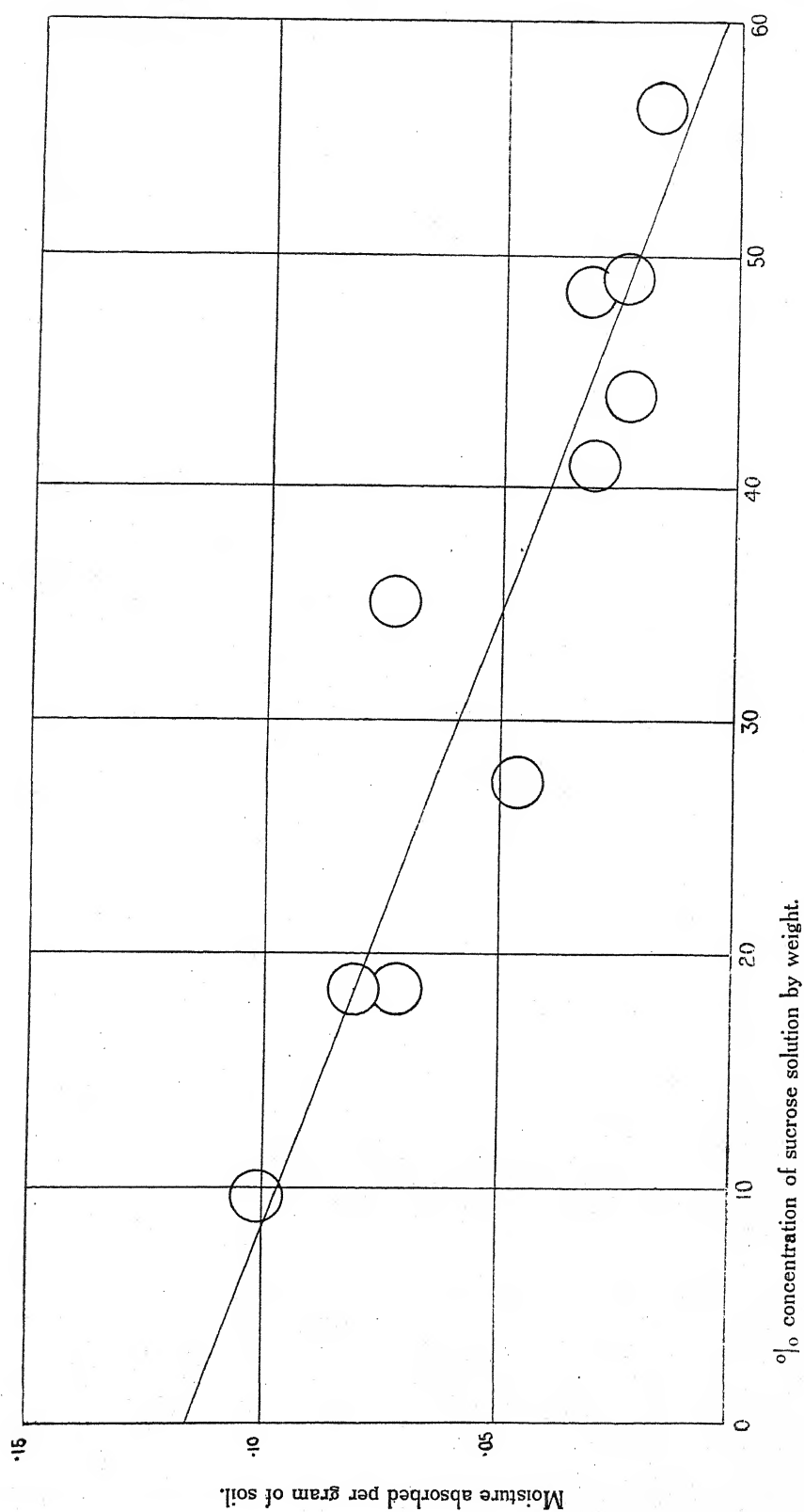


Fig. 11. Showing the absorption of water by soil in sugar solutions of various strengths.





the curve is extrapolated to zero concentration it is found that the soil absorbs 11.5 gm. of water per 100 gm. of soil.

The hygroscopic co-efficient of the soil, *i.e.*, the amount of water absorbed per 100 gm. soil when in equilibrium with a saturated atmosphere, is 2.43. One might expect that the amount of water absorbed by the soil colloids from a saturated vapour phase would be the same as that taken up from the pure solvent which is in equilibrium with the vapour, and theoretically, if no surface forces due to the state of division of the colloid are considered, this should be the case. Thus Alway (*J. A. R.*, Vol. VII, 350, 1916) states, "Theoretically, actual equilibrium would not be obtained until the moisture content of the soil equalled that of the same soil in actual contact with water, but the time required for this is so great that this theoretical consideration does not affect the present discussion." Actually, as these experiments show, the amount of moisture absorbed by the soil when in contact with the water is much greater than that absorbed from the vapour phase. The ratio of these amounts,  $11.5/2.43=4.73$ , is remarkably near the factor 4.26 in the Briggs-Shantz empirical formula connecting moisture-holding capacity of soils with their hygroscopic co-efficients.

This relation can be expressed as follows,

[Moisture-holding capacity] = [Hygroscopic co-efficient]  $\times 4.26 - 21.0$   
with which are to be compared the value of the factor (4.73) determined from the absorption experiments, and the value 23.46 deduced theoretically from equation (7) of §3.

If these results are confirmed by further work on different soils, it appears that we shall be in a position to form a much clearer idea of the true physical basis of these "constants." It can scarcely be accidental that both the theoretically deduced "free" moisture capacity of a soil and the experimentally determined ratio of the absorbed water to the hygroscopic co-efficient should agree so closely with the empirical equation evolved by the examination of a large number of soils. How far the specific character of the colloids of different soils will cause variations in the value of the factor from that calculated by Briggs and Shantz only further investigations can show.

With a knowledge of the variations of the "bound" moisture content of a soil with the osmotic pressure of the "free" solution in equilibrium with it, it will be possible to extend the calculation of "capillary" rise, given in §3.10, to the case of a soil containing a colloid. It is considered, however, that the curve produced above is not sufficiently accurate for the purpose, and, moreover, it will be necessary to examine the curve for clays contained in various soils of different geological origin before any generally applicable

relation can be deduced. The consideration of this point is, therefore, postponed.

From theoretical considerations it appears that if the equilibrium moisture content of a soil in contact with a liquid is not the same as that attained when contact is only with the vapour phase, which is itself in equilibrium with the liquid, the only explanation which will account for the fact is that a new phase is formed. Thus we may suppose that the hygroscopic co-efficient refers to the equilibrium of only two phases, colloid and vapour, while the moisture-holding capacity and the mechanical equivalent must be determined by the equilibrium of vapour, colloid, the free liquid phase and one other. It is suggested that besides the free water which will presumably obey the laws deduced in section (3) there is also what may be called vesicular water retained in the reticulated structure of the colloid.

In order to distinguish clearly between the states in which water exists "free" and "unfree," it will be as well to consider as "free" water only that which has a free surface; the remainder may be distinguished as "gel" water and vesicular water. At the hygroscopic coefficient a soil will thus only contain "gel" water. The factor (4.2) by which it is possible to calculate the total gel and vesicular water in equilibrium with water at atmosphere pressure may be referred to as the "vesicular co-efficient." The vesicular water must be maintained in equilibrium with the colloid, free water and vapour phases by the mechanical pressure exerted by the colloid structure, and moreover, if this colloid behaves as a semi-permeable membrane to dissolved matter, osmotic forces will also come into play. It is quite possible, of course, that this fourth water-containing phase is crystalline, but, if so, it does not seem probable that the factor would be constant for soils of diverse origin. Many interesting lines of investigation present themselves for the testing of this theory which it is hoped it may be possible to pursue in the near future.

#### (§6) CONCLUSION.

Although, it is not yet possible to test completely the theoretical deductions advanced in section (2), some tentative conclusions may be drawn, which are of interest in practical application to agriculture.

In the case of a soil containing no colloid, such as a coarse sand, the behaviour of the soil solution will be determined completely by the average size of the particles and the surface tension of the solution. The composition of the solution draining from such a soil should therefore remain unaltered.

If however a saline water is added to a soil containing colloid, water will tend to move through the colloid phase in order to dilute the solution if its

osmotic pressure is greater than the swelling pressure of the colloid, or the solution will become more concentrated if the reverse is the case. This is well illustrated by the following experiment :—

Two quantities of soil were made up to a moisture content of 7·5 and 5 per cent. and similar quantities with the same moisture contents but containing respectively 20 and 15 per cent. of solution of calcium ferrocyanide. These were pressed into slabs, made up in pairs and separated by parchment paper. The pairs of slabs were surrounded with tin foil and kept in airtight bottles. After various intervals of time a portion was cut off each slab and analysed. The results are shown below :—

Time	RATIOS OF MOISTURE IN SLABS SALT LAYER WATER LAYER		REMARKS
	7·5 per cent. moisture 20 per cent. salt	5 per cent. moisture 15 per cent. salt	
0 .. ..	1·0	1·0	....
4th day .. ..	1·20	1·10	No salt moved,
6th day .. ..	1·40	1·30	No salt moved,
8th day .. ..	1·30	1·20	Salt moved.

It thus appears that the first action is for free water to move across to the salt layer in order to dilute the solution, thus reducing the osmotic pressure and increasing the hydrostatic pressure on the water side. The salt then diffuses back carrying with it water. The final equilibrium will thus be obtained, in the absence of a semi-permeable membrane, when the salt solution is equally distributed throughout, but the rate at which the salt solution will move back may be very slow.

Since the equilibrium distribution of water in a soil is determined by the vapour pressure gradients in the soil atmosphere, the "bound" water of the colloid will have a definite value at equilibrium at each point in the column; the concentration of the sub-soil water will therefore affect the "capillary" rise, whereas, in the case of a sand, it will only do so in virtue of its concomitant variations in surface tension. Since therefore the solution draining from a soil may be supposed to be in virtual osmotic equilibrium with the colloid at each point, the composition of the solution will be determined by the colloid content. We should expect therefore that the ground water beneath a heavy soil would be more saline than in the case of a light soil.

Again, the rate of movement of a soil solution in a sandy soil should depend only on the gradient of hydrostatic pressure due to film tension, and the viscosity of the solution. In the case of a soil containing colloids, however, we have two rates to consider—the rate of movement of water in the colloid phase—and also the rate of movement of the free solution in virtue of its hydrostatic pressure.

The importance of considerations such as these in determining the advisability of shallow and frequent, or heavy and infrequent irrigations need not be emphasized. It would appear that in a light soil where the composition of the drainage water is unaffected, water economy should demand light and frequent waterings. In the case of a heavy soil however, owing to the possibility of the concentration of the soluble matter of the irrigation water by virtue of a rate of diffusion of the water through the colloid phase to drier parts of the soil, greater than that of the free solution, the soil may become saline; heavy waterings, therefore, seem indicated. Moreover, although in such a soil, the water may penetrate beyond root range, it will still be available to some extent to the crop by upward movement through the colloid phase without carrying up salt.

The above points are not advanced as definite conclusions but only as indications of lines of investigation which are opened up by a clearer consideration of the physical factors involved in the movement of a salt solution through a soil. Before, however, it is possible to deal with the dynamics of these questions, it is necessary to obtain all the information possible of their statics.

## SUMMARY.

1. The importance of studying all the factors affecting the movement of water in the soil and particularly the salts contained in the irrigation water, is pointed out.
2. An approximate calculation enables an estimate to be formed of the amount of water percolating from an irrigation area. Considerations of the residual sodium salts applied with the irrigation water and not removed by the crop enable rough conclusions to be drawn as to the average percolation from irrigated fields in the Lower Chenab canal colony.
3. Theoretical relations determining the equilibrium of a "free" solution having an osmotic pressure with the water "bound" in the colloid phase, and the vapour phase, are deduced by thermodynamical considerations.
4. From considerations of surface tension, a relation is deduced between the average size of soil particles and the film tension of water in the soil at various concentrations, by means of which it is possible to calculate the statical distribution of water in a column of soil saturated at its base.
5. The relation of these deductions to the empirical soil "constants" is discussed and a satisfactory result is obtained for the calculation of the moisture-holding capacity of soils.
6. Experimental results are given showing the variation of "bound" water in the colloid of a soil with the osmotic pressure of the solution. Extrapolation to zero osmotic pressure gives a value for the moisture absorbed by the colloid which is 4.7 times the hygroscopic coefficient. On the basis of these results an explanation of the empirical relationship between the moisture holding capacity of a soil and its hygroscopic coefficient is afforded.
7. It is suggested that the "bound" water of the soil exists in two phases which have been provisionally named "gel" water and vesicular water.
8. A preliminary experimental investigation of the compressibility of soils is described, but the results so far obtained do not allow of any deductions being made as to the swelling pressure of the soil colloids, for which purpose it was intended.



9. Experiments are described in which the hydrostatic pressure due to film tension is balanced against the osmotic pressure of a soil solution.

10. Certain tentative conclusions bearing on the composition of the solution draining from soils, and on the economic use of water are pointed out.

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# VARIATIONS IN SOME CHARACTERISTICS OF THE FAT OF BUFFALO AND COW MILK WITH CHANGES IN SEASON AND FEEDING.\*

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Most of the work published in India up to the present time on the composition of dairy products deals almost entirely with milk alone. In these publications<sup>1</sup> data regarding the proportion of total solids, fat, solids not fat, protein, sugar, etc., are given, and the milk of both buffaloes and cows has been investigated from this standpoint. In the chemical control of dairy products and in standardizing the same, certain characteristics of the fats based upon their chemical properties are used in most countries as a guide to the purity or otherwise of butter and similar substances. In India little data bearing upon this point have been published although some useful determinations with respect to *ghee* (clarified butter) have been published elsewhere<sup>2</sup>.

\* Paper read at the Seventh Indian Science Congress, Nagpur, January 1920.

<sup>1</sup> (a) *Mem., Dept. Agri. India, Chemical Series*, vol. II, nos. 2 and 4.

(b) *Analyst* (1901), vol. XXVI, p. 40.

<sup>2</sup> (a) *Jour. Asiatic Society of Bengal* (1910), vol. VI, 181.

(b) *Jour. Soc. Chem. Ind.* (1910), 1428.

(c) *Analyst* (1911), vol. XXXVI, p. 392.

(d) *Jour. Soc. Chem. Ind.* (1915), vol. XXXIV, p. 320.

(e) *Analyst* (1915), vol. XXXV, p. 343.

(f) *Jour. Soc. Chem. Ind.* (1917), vol. XXXVI, p. 118.

The chief analytical processes used in the examination of butter fat are the Reichert Meissl, the Polenske and the Kirschner while in recent years the Barium method of Ave Lallement (*vide* Bolton and Revi's Fatty Foods, p. 126) has assumed considerable importance. In addition, the simpler determinations of saponification and butyro refractometer readings are usually made. Briefly the Reichert Meissl number indicates the quantity of volatile fatty acids present, these acids being chiefly butyric and caproic. The Polenske process differentiates between the volatile fatty acids soluble and insoluble in water while the Kirschner number affords an indication of the butyric acid present. The Ave Lallement process is considered a valuable auxiliary to the Reichert Meissl process in deciding whether butter fat is adulterated or not and is based on the relative solubilities in water of the barium salts of the fatty acids.

For the purposes of food control many countries have fixed limits for certain analytical determinations within which limits the analytical figures of any samples of butter fat must fall in order for the latter to be passed as pure.

It is not necessary to specify in full what these standards are as they may be found in well known text books (*vide* Allen's *Commercial Organic Analysis*, Vol. II, p. 303). The Reichert Meissl figure is the one to which most importance is attached and the following minimum limits are taken in various countries. In England a Reichert Meissl figure below 24 is generally considered as indicating impure butter but the figure is not a legal standard. The United States of America have a legally recognized standard of 24 as a minimum. In Belgium, if the Reichert Meissl figure is below 28, the butter is considered adulterated, but one at least of certain other conditions must be simultaneously obtained. Italy recognizes butter fats with a Reichert Meissl figure above 26 as pure and below 20 as adulterated and casts suspicion on anything giving a figure coming between these limits. Sweden takes the minimum figure as 23. It is recognized however that under special conditions, particularly with a single animal, quite unusual figures can be obtained even with perfectly genuine butter fat.

The authors were able to obtain samples of genuine butter fat from animals fed under various conditions at the Telenkheri and Agricultural College Dairies, Nagpur. The milk was brought direct from the dairy at the time of milking to the laboratory where it was separated, butter prepared from the cream by hand-churning and butter fat in a pure dry condition obtained in the usual way. Of these samples there can be no doubt that they are the perfectly genuine production of milch animals.

When dealing with the supply of dairy products in India it must be remembered that unlike some countries milk from an individual animal is very frequently sold while mixed milk from herds is also distributed. The peculiarities of the individual animal must therefore be taken in consideration.

The authors have obtained the butter fat produced by an individual cow and a buffalo during the course of their lactation periods and also that produced by mixed herds of cows and buffaloes. They have made the usual physical and chemical examinations of these samples in order to determine to what extent perfectly genuine samples of butter fat can vary during changes in feeding and season.

The data available on the nature of the butter fat produced by Indian milch cattle are at present very small. Most of that published deals with *ghee* or clarified butter fat which in the process of manufacture is heated to a boiling point for a considerable time.

The samples dealt with in this investigation were heated to a temperature not above 60° C. in order to melt and filter off the liquefied butter fat. There is little evidence to show whether the differences in the methods of preparation of butter and *ghee* would affect the chemical and physical values.

The authors have made a few determinations on butter fat and on the *ghee* prepared from the same. The figures tabulated below (Table I) were obtained from which it will be seen that the process used in the manufacture of *ghee* does not apparently modify the chemical and physical characteristics to any important extent. This point will be further considered. It must be taken however that the figures quoted later apply to pure butter fat.

#### DISCUSSION OF RESULTS.

##### *Single buffalo (Table II).*

The saponification number was high at the commencement of the lactation period during the hot dry months from March to June. It fell slightly when green food was available during the monsoon and also when the weather was really cold in December and January. In sympathy with the high saponification value, a high negative value for the Ave Lallement determination was obtained although on one or two rare occasions this figure changed slightly to the opposite sign. The Reichert Meissl figure was high all through, but reached its lowest limit during the monsoon and cold months. The Polenske number was generally low compared with other published figures.

The butyro refractive index varied very little but increased towards the end of the lactation period. The iodine value is low but it may be noted that no linseed cake was fed during the course of the observation. It is useful to observe the rations fed during the lactation period.

Months	Bulky food	Concentrated food
March and April .. ..	Dry grass and green clover	<i>Tur chuni</i> , cotton seed, <i>til</i> cake or undecorticated cotton cake, with <i>juar</i> grain from middle of September.
May .. ..	Dry grass and silage	
June and middle of July ..	<i>Juar</i> fodder	
Middle of July to middle of August ..	Green grass	
Middle of August to December ..	Green <i>juar</i>	
December to end of lactation period ..	Dry grass and green clover	

*Daily ration.*

25 lb. green fodder plus 15 lb. dry fodder  
 or 35 „ green fodder alone .. ..  
 or 25 „ dry fodder alone .. ..

Plus 6 lb. (50 per cent. milk yield) concentrated food.

		lb.
<i>Chuni</i> ..	..	2
Cake ..	..	2
Cotton seed ..	..	1
<i>Juar</i> or cotton cake ..	..	1
TOTAL ..	..	6

*Single cow (Table III).*

The saponification numbers were generally lower than those obtained with the butter fat of the single buffalo. The same changes, *viz.*, high values at the commencement of the lactation period and low values during the monsoon months were observed. The Ave Lallement values were generally either slightly positive or slightly negative except early in the lactation period when high negative values were obtained.

The Reichert Meissl values were generally much lower than those obtained with the buffalo while the Polenske values were distinctly low all through and the same remark applies to the iodine values.

In some cases the Reichert Meissl value was lower than the minimum usually accepted in other countries; this was most noticeable in the monsoon months. The butyro refractive readings were higher than those given by buffalo butter fat.



*Rations fed during the course of observations.*

Months	Bulky food	Concentrated food
February, March to middle of April	Green clover	<i>Tur chuni</i> .. 1 lb.
Middle of April to middle of July	<i>Juar</i> fodder Silage and <i>juar</i> fodder ..	Cotton seed .. 1½ „ <i>Til</i> or cotton cake .. 1½ „ Cotton meal .. 1 „
Middle of July to middle of October	Green <i>sann</i> and <i>juar</i> fodder	After September 16th 1 lb. <i>juar</i> meal was given instead of 1 lb. cotton meal.
Middle of October to middle of December	Green <i>juar</i>	
Middle of December to end of lactation period	Clover and <i>juar</i> fodder.	

*Daily ration.*

20 lb. green fodder plus 10 lb. dry fodder	} Plus 5 lb. (40 per cent. milk yield) concen- trated food.
or 30 „ green fodder alone ..	
or 20 „ dry fodder alone ..	

*Herds of buffaloes and cows.*

The samples on which these determinations were made were taken from the Telenkheri Dairy Farm. Buffaloes and cows were fed on similar rations and as the observations were made over a complete year, the influence of the period of lactation of the individual animal was to a large extent eliminated. The herds may be said to have been fed in a manner common amongst professional dairymen.

The rations were as follows:—

Months	Bulky food	Concentrated food
February and March ..	<i>Juar</i> fodder, dry grass and clover ..	Cotton seed, <i>chuni</i> , gram <i>phol</i> , <i>Til</i> cake.
April .. ..	Dry grass and clover ..	Cotton seed, <i>chuni</i> , gram <i>phol</i> , wheat <i>bhusa</i> . <i>Til</i> cake.
May .. ..	<i>Sann</i> and maize (green) dry grass	<i>Chuni</i> , cotton seed, <i>til</i> cake, cotton cake (from 13th May).
June .. ..	<i>Sann</i> and <i>juar</i> (green) ..	Cotton seed, <i>chuni</i> , cotton cake, <i>til</i> cake, linseed cake (26th to 30th only).
July .. ..	Green <i>juar</i> .. ..	Cotton seed, <i>chuni</i> , <i>til</i> cake, linseed cake, cotton cake (1st to 11th).
August .. ..	Green <i>juar</i> .. ..	Cotton seed (1st to 9th), <i>chuni</i> , linseed cake.



Months		Bulky food		Concentrated food
September	..	Green grass	..	<i>Til</i> and linseed cake, <i>chuni</i> (1st to 5th and 26th to 30th).
October	..	Do.	..	<i>Chuni</i> , <i>til</i> and linseed cake.
November	..	Do., dry grass	..	Ditto
December	..	Green grass, clover, dry grass	..	<i>Chuni</i> , <i>til</i> cake, linseed cake, cotton seed from 20th to 31st.
January	..	Clover and dry grass	..	<i>Chuni</i> , cotton seed, <i>til</i> cake, <i>tur phol</i> from 9th to 31st. Linseed cake (1st to 17th).
February	..	Clover, dry grass	..	<i>Chuni</i> (1st to 20th), <i>tur phol</i> (1st to 25th), <i>til</i> cake.
March	..	Clover and dry grass	..	<i>Chuni</i> (20th to 31st), cotton seed, cotton cake (20th to 31st). Linseed cake, <i>til</i> cake.
April	..	Clover and <i>juar</i> fodder	..	<i>Chuni</i> , cotton cake, linseed and <i>til</i> cake.

*Approximate daily rations.*

15 to 20 lb. green fodder plus 20 to 25 lb. dry fodder plus concentrated food equivalent to 60 per cent. of the milk yield, the various concentrates being mixed in equal proportions.

MIXED BUTTER FAT FROM A HERD OF BUFFALOES (*Table IV*).

The outstanding features of these determinations are the low figures obtained for saponification, Reichert Meissl, Polenske and Kirschner numbers. The Ave Lallement determinations gave generally fairly high positive values. It is worthy of note that the lowest values were obtained during the hot dry months. Judged by the usual standards, the butter fats produced during these months by this herd would be classed in most countries as adulterated. Only during the months when green fodder was available, did the figures for Reichert Meissl number approximate to the standards of other countries. The iodine values obtained in these determinations were fairly high particularly during the months when linseed cake was fed. During those months, however, the melting point of the fat was low—a disadvantage from a commercial dairyman's point of view.

MIXED BUTTER FAT FROM A HERD OF COWS (*Table V*).

The remarks made upon the figures obtained from the butter fat from a herd of buffaloes apply equally well to those obtained from cows. Very low

values for saponification, Reichert Meissl, Polenske, etc., are characteristic of the dry months. The Ave Lallement figure was generally positive. The butyro refractometer readings for butter fat from buffaloes and cows fed under the same conditions were similar. The melting point of butter fat from cows was rather lower than that from buffaloes.

The thanks of the authors are due to Mr. Ram Narayan, B. Ag., for assistance in the analytical work and also to the officers in charge of the Telenkheri and Agricultural College Dairies, Nagpur.

### SUMMARY.

I. The authors have made a series of determinations of the physical and chemical characteristics of pure butter fat obtained from cows and buffaloes fed under known conditions.

Observations were made both on single animals and on herds.

II. Single animals can produce a butter fat which gives figures widely different from those of a herd.

III. Variations from the figures usually obtained in other countries are particularly noticeable in the case of saponification, Reichert Meissl, Polenske and Ave Lallement values.

IV. If judged by standards usually accepted in other countries, much of the butter fat produced by Indian milch cattle would be pronounced as adulterated.

V. In the case of herds, the departure from usually accepted figures is greatest during the months when hot dry weather prevails.

VI. The usual chemical and physical determinations made on butter fat do not differentiate between the butter fat produced by cows or buffaloes.

TABLE I.

	Saponi- fication No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallement	Melting point
Pure butter fat ..	216.1	38.92	22.90	0.88	19.44	+11.8	42.9
Ghee prepared from above..	216.1	38.38	21.50	0.66	19.72	+7.3	43.5
Pure butter fat ..	218.6	38.47	22.32	1.15	18.96	+9.0	42.7
Ghee prepared from above..	219.1	38.00	22.09	1.07	19.26	-3.6	42.5

TABLE II.  
*Lendra single buffalo.*

*Date of calving—17-2-14.*

*Experiments started from 12-3-14.*

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallément	Butyro refractive index at 40°C.	Melting point
1916-17									
March to April 1 to 8	Evening ..	236.8	25.0	33.36	1.37	31.67	-42.0	41.3	...
		247.6 228.5	30.7 20.8	38.47 25.80	1.46 1.26	33.90 29.64	-65.0 -25.5	42.3 40.7	...
	Morning ..	235.9	26.1	35.90	1.10	31.69	-62.8	40.9	...
		241.0 232.3	28.9 23.4	36.98 34.38	1.17 1.02	32.09 31.29	-114.8 -29.8	41.3 41.0	...
April to May 9 to 16	Evening ..	233.4	25.3	36.89	1.28	32.85	-17.8	40.85	...
		234.8 231.5	26.5 24.1	40.35 34.83	1.09 1.02	36.65 30.76	+17.7 -30.8	41.20 40.50	...
	Morning ..	231.8	25.9	38.65	1.74	32.12	-20.5	40.88	...
		236.6 226.3	26.8 25.0	39.60 38.09	2.48 1.41	36.14 34.10	-12.9 -31.2	41.10 40.70	...
May to June 17 to 24	Evening ..	233.3	25.1	37.52	1.65	32.85	-41.5	40.75	...
		234.0 232.3	25.5 24.2	39.05 34.57	1.85 1.36	34.50 30.46	-21.4 -70.4	40.90 40.70	...
	Morning ..	233.4	23.0	37.99	2.23	33.63	-65.1	40.75	...
		236.0 231.4	24.2 21.9	41.42 34.91	3.36 1.46	37.40 29.65	-24.5 -123.2	41.20 40.00	...

June 25 to 32	Evening ..	229-8	25-8	37-01	1-42	32-35	-49-8	40-90	....
	Morning ..	231-4 227-4	26-9 23-9	37-86 35-83	1-96 1-17	32-98 31-51	-13-7 -97-8	41-50 40-40	....
July 33 to 40	Evening ..	229-8	25-7	36-33	1-59	32-11	-54-3	41-35	....
	Morning ..	232-5 224-7	27-2 22-1	39-59 30-71	1-81 1-08	35-80 26-96	-7-5 -60-0	41-80 41-00	....
August 41 to 48	Evening ..	227-4	23-4	32-23	1-00	28-75	-36-3	42-1	....
	Morning ..	231-4 222-3	24-8 21-3	35-65 27-21	1-12 0-78	31-31 24-70	-39-6 +34-3	43-2 41-8	....
September 49 to 56	Evening ..	228-3	26-8	32-39	1-27	27-64	-68-2	41-7	....
	Morning ..	229-7 227-4	31-4 19-9	33-82 31-43	1-32 1-17	29-42 25-71	-41-8 +85-9	42-3 41-4	....
October 57 to 64	Evening ..	228-6	25-2	32-01	1-12	28-05	-56-3	42-5	....
	Morning ..	230-4 223-4	26-7 23-3	33-48 32-26	1-21 0-91	29-33 25-78	-61-5 -50-3	42-8 42-0	....
November 65 to 72	Evening ..	228-8	23-3	30-80	1-29	27-30	-55-5	42-6	....
	Morning ..	230-3 225-5	25-4 22-0	33-51 27-83	1-44 1-11	29-10 25-90	-17-2 -93-1	43-4 41-8	....
December 73 to 80	Evening ..	228-0	25-3	31-65	1-23	27-33	-30-0	42-9	....
	Morning ..	232-1 228-1	26-7 24-1	32-60 29-12	1-51 1-05	28-92 23-82	-44-4 -16-6	43-6 42-3	....
January 81 to 88	Evening ..	228-0	23-3	32-77	1-33	28-85	-16-1	42-7	....
	Morning ..	231-1 224-5	24-4 22-4	35-47 29-62	1-64 1-19	31-47 26-31	-11-1 -17-1	43-2 42-6	....
February 89 to 96	Evening ..	228-9	23-2	31-70	1-37	27-66	-39-3	42-6	....
	Morning ..	230-3 228-1	27-3 20-7	32-72 30-27	1-57 1-15	28-19 26-88	+0-8 -75-0	42-9 42-1	....
March 97 to 104	Evening ..	232-4	24-1	34-33	1-49	29-40	-38-4	42-2	....
	Morning ..	233-7 231-8	26-5 22-5	34-60 33-89	2-05 1-20	30-69 28-88	-15-0 -64-6	42-6 41-9	....

TABLE II—(concl'd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallment	Butyro refractive index at 40°C.	Melting point
October to November 65 to 72	Evening ..	232.5	24.7	33.26	1.75	29.05	-45.1	42.4	...
		234.8 228.3	24.8 22.0	35.37 31.63	2.15 1.70	31.17 27.27	-82.0 -25.3	42.6 40.9	...
	Morning ..	235.1	22.2	34.08	1.76	30.26	-83.9	41.3	...
		237.6 233.2	27.0 19.0	35.94 33.72	1.93 1.59	32.27 28.94	-89.8 -78.1	41.9 40.9	...
November to December 73 to 80	Evening ..	234.4	23.2	36.5	2.27	31.62	-38.4	41.1	..
		237.9 225.8	26.5 21.1	38.7 34.7	2.82 1.74	33.34 30.22	-10.3 -79.8	41.3 41.0	...
	Morning ..	235.0	24.6	36.3	2.35	31.84	-50.5	40.9	...
		238.7 225.3	25.6 23.0	37.8 33.5	3.02 1.72	32.72 30.95	-29.5 -65.4	41.3 40.4	...
December to January 81 to 88	Evening ..	225.6	30.2	31.85	1.24	27.84	-9.5	42.6	...
		231.5 217.9	32.2 28.2	33.68 30.19	1.52 1.00	28.88 27.07	+1.8 -24.4	43.2 41.7	...
	Morning ..	229.9	29.7	31.24	1.21	27.24	-9.7	42.8	...
		231.8 228.2	32.1 28.0	33.29 29.54	1.26 1.17	28.81 25.54	15.7 -17.2	43.0 42.2	...
January to February 89 to 96	Evening ..	227.3	32.1	28.80	1.20	26.83	-64.0	43.0	...
		229.2 225.8	34.5 29.9	31.25 26.43	1.28 1.12	27.39 26.32	-11.3 -107.0	43.5 42.6	...
	Morning ..	229.8	29.3	29.80	1.24	27.63	-69.2	43.0	...
		232.0 228.4	31.9 26.4	32.08 27.40	1.48 1.06	28.42 27.14	-25.0 -96.9	43.9 42.6	...



TABLE III.  
Lendra single cow.  
Experiments started from 14-2-16.

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallament	Butyro refractive index at 40°C	Melting point
1916-17									
February to March 1 to 8	Morning ..	237.6	25.18	32.33	1.38	27.03	-59.2	44.6	39.7
		241.3 229.2	27.99 23.66	33.85 31.03	1.64 1.06	29.21 26.18	-70.0 -22.7	45.4 43.6	40.1 39.5
	Evening ..	230.2	26.03	29.05	1.02	25.09	-35.0	45.3	39.1
		234.4 225.6	29.94 20.90	29.51 28.36	1.11 0.93	25.19 24.96	-58.2 -18.2	46.1 44.7	40.3 37.5
March to April 9 to 16	Morning ..	233.4	27.20	31.62	1.31	26.35	-41.4	45.3	39.1
		240.3 229.3	31.34 22.84	34.05 29.69	1.53 1.09	28.79 25.17	-79.0 -19.0	46.3 43.4	40.9 37.7
	Evening ..	223.6	30.04	26.21	1.03	23.38	-8.3	45.0	38.8
		225.4 224.1	32.47 27.64	29.93 24.03	1.17 0.90	25.57 21.00	-22.3 5.0	45.6 44.3	39.7 37.7
April to May 17 to 24	Morning ..	227.4	24.99	30.48	1.19	25.36	-8.5	43.4	37.8
		228.9 224.3	34.0 18.98	31.64 27.61	1.30 0.96	26.82 23.26	-9.9 -2.5	44.4 43.2	38.7 36.9
	Evening ..	229.6	24.39	30.15	1.10	25.64	-22.4	44.0	39.8
		234.8 226.5	26.06 22.91	32.35 28.24	1.17 0.93	27.44 24.17	-58.3 -7.5	45.1 42.8	40.7 38.9



TABLE III—(contd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallement	Butyro refractive index at 40°C.	Melting point
May to June 25 to 32	Morning ..	226.6	28.14	29.49	1.06	25.19	-4.3	43.3	39.3
		225.8 228.1	30.51 25.86	30.58 28.92	1.26 0.99	26.33 24.75	-8.3 -0.1	43.8 42.8	40.1 38.3
	Evening ..	225.2	25.58	28.54	1.16	24.50	-2.3	43.3	39.2
		226.6 223.6	32.44 19.72	29.77 27.11	1.39 1.00	25.66 23.28	-5.2 -0.3	43.7 43.1	39.7 38.1
June 33 to 40	Morning ..	223.7	28.50	27.00	1.32	23.08	+2.9	43.8	39.7
		225.6 222.3	33.31 21.90	28.70 25.30	1.61 1.18	25.08 21.08	+7.3 -1.0	44.3 43.2	40.3 38.9
	Evening ..	223.3	28.09	26.58	1.16	22.72	+2.0	44.4	39.6
		226.5 222.1	33.78 24.14	29.43 24.60	1.33 1.00	24.78 21.31	+4.4 -1.6	44.8 40.8	41.1 37.7
July 41 to 48	Morning ..	221.0	27.40	25.51	0.98	21.84	+0.5	43.9	42.2
		226.6 219.0	32.62 24.21	28.41 24.43	1.30 0.78	23.99 20.67	+4.9 -5.3	44.8 42.5	44.1 39.9
	Evening ..	220.3	26.35	23.85	0.84	20.53	+5.8	44.5	41.2
		221.0 219.4	33.61 19.28	25.13 23.09	0.84 0.83	21.30 19.84	+7.4 -3.1	45.1 44.1	44.3 38.7
July to August 49 to 56	Morning ..	218.7	27.93	24.53	0.82	21.64	+3.2	43.5	41.6
		223.6 214.6	29.20 25.78	27.44 22.70	1.00 0.68	23.40 19.59	+13.6 -1.7	44.6 42.8	45.0 39.9
	Evening ..	220.3	24.12	22.77	0.72	19.55	-5.5	44.4	41.7
		224.5 214.4	28.20 20.36	26.45 20.73	0.89 0.59	20.40 18.49	+11.9 -3.61	46.5 42.1	43.7 40.3

August to September 57 to 64	Morning ..	219.4	27.32	24.05	0.80	21.18	+1.5	43.9	40.3
	Evening ..	221.9 215.3	38.10 20.45	25.36 22.93	0.96 0.72	22.63 20.22	+6.5 -3.0	44.4 43.0	44.1 36.7
September to October 65 to 72	Morning ..	221.7	26.28	24.42	0.91	20.78	+3.8	43.3	40.8
	Evening ..	222.4 221.1	32.81 32.40	25.39 23.49	1.15 0.75	21.51 19.76	+11.0 -0.8	43.7 43.4	42.1 39.7
October to November 73 to 80	Morning ..	223.7	30.36	26.10	0.92	22.53	+0.65	42.4	42.7
	Evening ..	225.5 222.2	33.37 28.20	28.42 24.31	1.06 0.81	24.49 20.91	+6.0 -4.2	42.9 42.0	44.5 40.7
November to December 81 to 88	Morning ..	223.5	29.05	26.40	0.99	22.62	+3.05	42.1	41.9
	Evening ..	224.8 223.0	32.81 27.62	26.92 25.66	1.05 0.88	23.18 22.00	+4.6 +0.1	42.7 41.6	44.3 38.9
December to January 89 to 96	Morning ..	221.2	33.37	25.22	0.93	21.44	+6.5	43.0	41.7
	Evening ..	222.6 218.4	36.72 32.46	27.63 23.00	1.12 0.82	22.95 19.69	+12.1 +1.3	41.5 42.4	43.1 39.5
	Morning ..	219.6	32.08	24.10	0.80	20.85	+7.2	43.2	43.6
	Evening ..	221.9 218.3	38.85 26.63	25.06 23.27	0.96 0.71	21.74 20.03	+9.5 +6.0	44.2 42.4	47.6 40.5
	Morning ..	223.7	29.23	26.56	0.97	22.94	+4.6	42.2	43.1
	Evening ..	223.0 224.4	33.31 24.88	27.69 25.72	1.13 0.85	24.03 21.52	+5.7 +3.6	42.8 41.4	46.4 39.9
	Morning ..	223.9	30.64	26.26	0.92	22.51	+4.1	42.6	42.2
	Evening ..	225.0 223.2	31.86 28.51	26.72 25.53	0.99 0.80	23.02 21.74	+6.8 +2.9	43.0 42.2	43.7 41.3
	Morning ..	226.1	30.91	27.18	1.52	22.79	-1.0	42.6	41.3
	Evening ..	229.9 223.2	33.78 29.11	27.60 26.79	1.33 0.99	22.99 22.60	+4.3 -1.7	43.5 42.1	42.9 39.3
	Morning ..	223.9	32.83	25.23	1.25	21.45	+6.9	43.3	40.9
	Evening ..	223.1 221.0	36.53 30.71	26.93 23.78	1.10 0.82	22.69 20.48	+10.0 +4.3	43.9 42.7	43.1 38.5

TABLE III—(concl.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallemand	Butyro refractive index at 40°C.	Melting point
January to February 97 to 104	Morning ..	223.4	34.25	24.78	1.13	21.14	+9.2	43.7	40.9
		227.8 217.2	39.79 29.98	26.66 21.70	1.39 0.98	23.23 18.87	+16.9 +3.1	44.9 42.8	42.5 38.5
	Evening ..	223.1	34.74	24.07	1.04	20.76	+10.1	43.8	41.35
		226.4 217.5	36.92 29.69	25.36 21.10	1.35 0.82	22.04 18.31	+15.7 +6.4	44.5 42.5	42.5 39.1

TABLE IV.  
*Telenkheri herd of buffaloes.*

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijk No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallament	Butyro refractive index at 40°C.	Melting point
1916-17 March to April 1 to 8	Morning ...	212-0	38-08	19-45	0-54	17-88	+ 18-1	45-7	44-0
	Evening ..	212-7 211-6 213-2	39-34 37-77 39-11	19-78 19-25 19-51	0-67 0-47 0-59	18-10 17-90 18-00	+18-9 +16-4 +17-2	46-4 45-0 45-6	45-4 44-1 44-2
April to May 9 to 16	Morning ..	214-6 212-2	40-06 38-20	20-16 19-18	0-62 0-55	8-67 17-59	+20-6 +13-1	45-7 45-4	45-4 43-3
	Evening	212-1 213-4 210-8 213-1	38-91 39-00 38-83 39-17	19-50 20-60 18-42 20-02	0-62 0-91 0-46 0-54	17-98 18-93 16-90 18-16	+17-3 +19-9 +14-9 +14-6	45-4 45-4 45-3 45-5	43-9 44-7 43-1 43-7
May to June 17 to 24	Morning ..	213-0 214-1 212-1	39-50 40-55 37-74	19-46 19-75 19-13	0-63 0-82 0-34	17-54 18-20 16-24	+15-3 +17-6 +12-4	45-3 45-7 45-2	43-8 44-3 43-2
	Evening ..	212-1 213-9 210-8	40-05 41-22 38-61	18-72 19-36 18-02	0-48 0-56 0-42	17-65 18-19 17-19	+17-9 +21-1 +15-7	45-6 45-9 45-4	44-6 45-8 43-4
June 25 to 32	Morning ..	213-2 214-1 212-3	39-63 41-7 38-14	18-98 19-60 18-43	0-71 1-06 0-49	17-12 18-11 16-65	+14-9 +18-6 +12-5	45-6 46-3 44-8	43-9 44-7 42-3
	Evening ..	214-4 215-9 212-5	39-50 40-52 38-09	18-89 20-19 18-08	0-53 0-70 0-33	17-45 18-69 16-71	+14-1 +18-2 +10-2	45-7 46-2 45-2	44-6 45-0 44-3

TABLE IV—(contd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallément	Butyro refractive index at 40°C.	Melting point
July 33 to 40	Morning ..	216.7	39.80	21.75	0.71	19.57	+ 7.1	46.2	43.3
		218.0 215.7	40.26 39.19	23.82 19.66	0.89 0.56	21.42 17.86	+12.1 +2.6	46.4 46.0	45.1 42.1
	Evening ..	215.6	42.39	21.02	0.68	19.04	+10.2	46.4	42.5
		217.1 214.5	43.70 41.43	22.51 19.31	0.80 0.58	20.29 17.35	+15.5 +7.3	46.7 46.0	43.5 41.5
July to August 41 to 48	Morning ..	217.1	38.89	23.39	0.75	21.07	+8.5	45.7	42.6
		217.4 216.8	39.60 38.27	24.06 22.27	0.87 0.70	21.92 19.96	+10.5 +5.5	45.8 45.5	43.0 42.5
	Evening ..	218.0	38.68	24.41	0.89	21.73	+5.3	45.6	41.5
		220.0 217.0	40.82 37.45	26.15 23.49	1.07 0.76	23.09 21.03	+8.7 +3.1	45.8 45.1	42.5 40.3
August to September 49 to 56	Morning ..	216.7	42.21	23.99	0.63	21.39	+3.8	46.0	42.8
		219.5 213.8	43.34 40.86	27.10 22.25	0.72 0.45	24.04 19.96	+9.5 -7.5	47.0 45.2	43.3 42.3
	Evening ..	217.4	42.46	24.46	0.75	21.40	+5.1	45.6	42.0
		220.5 213.7	43.97 40.99	27.86 21.55	0.90 0.52	24.79 18.84	+10.5 -2.5	46.4 45.3	44.1 41.1
September to October 57 to 64	Morning ..	219.4	41.48	27.44	0.77	24.42	+2.1	44.9	41.5
		223.2 215.0	43.81 39.60	30.64 24.33	0.87 0.59	27.31 22.20	+6.9 -2.0	45.8 44.0	43.1 39.7
	Evening ..	219.2	42.73	26.01	1.02	22.52	+3.4	45.2	40.8
		220.8 216.8	43.77 41.99	27.10 24.50	1.19 0.70	* 23.01 21.88	+8.8 -1.2	45.6 44.8	42.7 37.7



October to November 65 to 72	Morning ..	220-3	40-50	27-14	0-73	24-05	-1-8	44-9	41-2
	Evening ..	224-1 218-7 221-6 224-6 219-7	42-47 38-10 40-89 41-20 40-16	30-11 25-88 27-95 29-04 26-74	1-03 0-58 0-84 1-04 0-72	26-26 22-93 24-59 25-33 23-25	+2-1 -8-9 -2-4 +1-5 -7-2	45-3 44-3 45-1 45-4 44-8	42-1 40-2 40-7 41-1 39-9
November to December 73 to 80	Morning ..	219-1	41-38	25-82	0-64	22-98	+4-4	45-3	41-6
	Evening ..	220-1 218-3 219-3 220-5 216-9	42-73 40-31 41-34 43-83 40-37	27-32 24-6 25-27 26-80 22-39	0-78 0-54 0-77 0-87 0-62	24-10 21-85 22-50 23-95 19-84	+7-7 +2-7 +6-5 +10-7 +4-6	45-8 44-8 45-4 46-1 44-8	42-1 40-9 41-9 42-9 40-1
December to January 81 to 88	Morning ..	220-7	40-04	26-12	0-80	22-96	+1-8	44-8	42-0
	Evening ..	223-0 219-4 219-3 221-0 218-1	41-37 38-65 41-01 41-70 40-41	27-35 24-39 25-31 25-63 24-70	0-99 0-67 0-76 1-09 0-60	23-99 22-36 22-70 23-05 22-37	+2-7 +0-8 +4-8 +7-1 +2-3	45-2 44-5 45-1 45-3 44-8	42-9 40-9 41-7 42-7 40-3
January to February 89 to 96	Morning ..	219-5	40-18	24-35	0-73	21-61	+3-9	45-1	40-5
	Evening ..	222-1 215-8 220-2 225-2 216-8	41-86 37-76 40-97 43-03 39-46	24-91 23-46 24-63 27-53 22-69	0-79 0-69 0-68 0-76 0-61	22-06 21-13 21-99 24-55 20-37	+0-4 +0-2 +3-6 +10-6 -7-1	45-8 44-3 44-4 45-7 44-1	41-5 39-5 41-3 43-5 39-7
February to March 97 to 104	Morning ..	214-3	41-73	21-64	0-60	19-76	+12-1	45-6	42-9
	Evening ..	215-9 212-7 214-7 216-2 212-8	43-72 39-62 40-37 43-13 33-13	23-68 19-96 21-34 22-15 19-78	0-66 0-49 0-55 0-59 0-50	21-37 18-20 19-50 20-05 18-39	+16-3 +9-3 +12-9 +18-5 +9-5	45-8 45-3 45-5 45-8 45-1	43-9 41-9 43-1 44-5 40-7

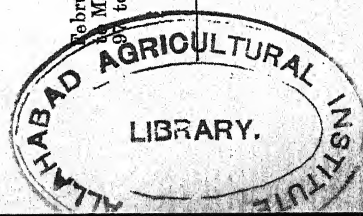




TABLE IV—(concl'd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallément	Butyro refractive index at 40°C.	Melting point
March to April 105 to 112	Morning ..	217.1	41.55	23.81	0.57	21.77	+8.1	45.0	41.9
		218.8 215.4	42.73 39.89	26.23 22.20	0.61 0.55	23.79 20.52	+11.6 +5.6	45.2 44.8	43.5 40.7
	Evening ..	215.6	41.78	22.73	0.64	20.66	+9.3	45.4	42.3
		217.1 214.7	43.39 39.93	23.39 22.19	0.80 0.56	21.44 20.00	+12.7 +8.1	45.8 44.9	42.8 41.5
April 113 to 119	Morning ..	212.2	42.55	20.65	0.64	18.97	+7.5	45.1	43.7
		212.9 218.8	43.07 42.03	21.21 20.18	0.69 0.56	19.12 18.79	+9.1 +5.1	45.3 44.9	44.5 43.1
	Evening ..	211.9	45.09	19.44	0.61	18.33	+14.7	45.4	42.4
		213.9 210.3	46.22 43.29	21.07 17.13	0.64 0.57	19.45 17.43	+17.5 +12.2	45.5 45.2	42.9 42.1

TABLE V.  
*Telenkheri herd of cows.*

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijs No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lalllement	Butyro refractive index at 40°C.	Melting point
1916-17 March to April 1 to 8	Evening .. {	213.8	39.8	18.79	0.71	16.22	+14.5	46.1	43.9
		214.3 213.3	41.15 36.91	19.94 18.25	0.78 0.55	16.65 15.75	+19.7 +10.0	47.1 45.7	46.2 41.5
	Morning .. {	215.3	30.00	19.84	0.86	17.24	+8.6	45.6	41.1
		216.4 214.4	40.56 39.44	20.99 18.58	1.03 0.77	18.01 15.90	+14.8 +9.4	45.9 45.3	41.3 40.9
April to May 9 to 16	Evening .. {	215.9	39.99	20.13	0.79	17.23	+14.7	45.5	40.6
		216.5 214.8	40.76 38.46	21.17 19.54	0.85 0.63	18.44 16.67	+17.9 +10.9	45.8 45.0	41.5 39.7
	Morning .. {	216.1	38.60	19.92	0.65	17.12	+14.8	45.6	42.0
		217.0 215.0	39.90 37.46	20.59 19.35	0.75 0.55	17.51 16.64	+15.9 +13.9	45.4 45.3	43.7 41.9
May to June 17 to 24	Evening .. {	214.3	38.81	19.23	0.64	16.64	+16.7	44.8	42.1
		215.8 212.4	39.58 37.84	20.43 18.21	0.76 0.61	17.74 15.75	+10.9 +13.17	45.4 44.2	42.9 40.6
	Morning .. {	219.9	37.56	19.55	0.76	16.89	+13.4	44.8	41.1
		216.7 213.3	39.82 33.21	20.58 18.23	0.97 0.61	18.02 15.99	+16.3 +8.4	45.4 44.3	41.6 40.6
June 25 to 32	Evening .. {	213.5	42.06	18.65	0.81	15.88	+18.1	45.4	41.4
		215.4 211.0	43.90 40.41	19.70 17.39	0.98 0.69	16.55 14.94	+21.6 +15.3	45.9 44.8	42.9 40.6
	Morning .. {	214.7	40.79	19.15	0.82	16.57	+17.6	45.2	42.3
		215.3 213.9	42.12 40.16	20.26 17.88	0.88 0.75	17.57 15.59	+22.0 +14.9	45.7 44.9	43.5 41.7

TABLE V—(Concl'd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wijk. No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallément	Bulryo refractive index at 40°C	Melting point
July 33 to 40	Evening ..	215.8	43.82	20.70	0.98	17.52	+12.6	45.8	39.1
		217.1 213.3	45.75 40.96	22.59 18.78	1.20 0.71	18.60 16.20	+15.7 +9.9	46.3 45.1	39.3 38.9
	Morning ..	218.6	41.95	22.56	1.22	18.59	+11.1	45.5	39.9
		219.0 217.8	44.92 37.91	23.54 20.85	1.41 0.94	19.20 17.44	+12.6 +9.9	45.6 45.4	40.1 39.7
July to August 41 to 48	Evening ..	217.8	41.0	22.80	1.26	18.82	+10.1	45.5	39.5
		218.7 216.1	42.07 39.90	23.92 21.14	1.67 1.08	19.54 17.47	+12.7 +5.7	45.6 45.2	40.3 38.7
	Morning ..	220.2	37.51	24.35	1.29	20.14	+5.6	44.6	40.3
		220.5 219.8	37.79 37.10	24.83 23.72	1.46 1.16	20.27 19.83	+7.6 +3.6	44.9 44.4	40.5 39.9
August to September 49 to 56	Evening ..	220.85	40.79	25.13	1.15	20.76	+2.7	44.7	38.1
		222.8 219.4	42.71 40.06	27.08 23.24	1.30 0.93	22.32 18.67	+8.0 -0.9	45.1 44.5	39.9 36.5
	Morning ..	222.40	38.87	25.45	1.16	20.00	+3.5	44.3	39.1
		224.8 219.8	40.40 37.50	26.87 24.27	1.48 0.78	21.35 19.22	+5.4 +1.3	44.9 43.5	41.9 36.6
September to October 57 to 64	Evening ..	223.3	40.4	25.96	1.08	20.88	-0.4	44.5	38.8
		224.5 219.6	43.95 37.50	27.80 23.84	1.36 0.83	21.86 19.33	+8.6 -6.3	45.2 43.8	41.5 37.5
	Morning ..	223.5	37.0	26.39	1.44	21.14	-1.0	44.0	38.8
		224.5 223.0	37.56 36.45	27.40 24.80	1.57 1.24	22.21 19.88	+4.6 -5.2	44.2 43.6	40.5 38.0

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October to November 65 to 72	Evening ..	222-1	39-93	25-00	1-15	20-07	+6-4	44-5	37-95
		223-2 221-1	40-51 39-32	26-36 24-08	1-32 1-00	20-33 19-90	+9-1 +4-7	44-7 44-2	38-9 36-9
	Morning ..	221-7	37-97	25-59	1-23	20-75	+1-7	44-3	38-80
		224-3 218-8	39-53 35-82	26-32 24-89	1-48 0-87	21-70 20-09	+6-9 -2-7	44-6 44-1	40-5 37-5
November to December 73 to 80	Evening ..	219-2	42-21	23-58	0-93	19-17	+7-1	44-8	39-2
		221-9 217-3	43-36 41-09	25-05 22-76	1-08 0-71	19-48 18-45	+11-0 +3-3	45-3 44-6	40-7 37-3
	Morning ..	221-2	40-61	24-29	1-04	19-58	+6-4	44-5	38-1
		222-5 219-8	41-40 39-85	25-34 23-11	1-17 0-77	20-40 18-99	+9-3 +3-9	45-0 43-9	38-5 37-2
December to January 81 to 88	Evening ..	220-0	40-22	23-36	1-13	19-88	+9-4	44-5	38-8
		224-0 217-3	42-00 38-64	24-51 22-41	1-34 0-90	19-83 18-41	+11-7 +4-5	44-7 44-3	39-3 37-5
	Morning ..	222-3	38-33	24-56	1-38	19-60	+6-7	44-2	38-1
		224-5 221-2	39-24 37-53	24-93 24-31	1-51 1-08	19-91 19-17	+7-6 +5-3	44-5 43-7	39-3 36-9
January to February 89 to 96	Evening ..	222-1	39-53	23-73	1-35	18-98	+8-7	44-7	38-8
		226-1 218-0	41-07 38-74	25-11 22-10	1-49 1-29	19-96 18-50	+15-8 +2-2	45-2 44-2	41-1 36-7
	Morning ..	222-6	39-78	23-74	1-24	18-47	+7-2	44-5	37-4
		225-0 219-8	40-65 39-23	24-64 22-33	1-40 1-01	18-82 17-85	+12-0 +3-1	44-7 44-3	38-3 36-7
February to March 97 to 104	Evening ..	217-1	42-6	21-60	0-86	18-30	+10-8	45-3	39-2
		217-9 216-2	43-8 41-8	22-24 21-25	1-02 0-68	18-86 17-70	+13-7 +6-9	45-6 45-0	39-9 38-5
	Morning ..	219-4	39-8	23-70	1-02	19-38	+7-2	44-6	38-4
		221-0 217-5	40-9 38-2	23-82 23-54	1-14 0-80	20-30 18-58	+12-3 +4-1	44-9 44-2	39-5 37-9

PART V—(concl'd.)

Month and numbers of samples	Time of milking	Saponification No.	Iodine Wjfs' o.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lallement	Butyro refractive index at 40°C.	Melting point
March to April 105 to 112	Evening ..	215.0	43.57	20.58	0.74	17.79	+14.1	45.5	38.4
		216.5 214.1	45.03 45.92	21.50 19.67	0.87 0.68	18.45 17.38	+18.2 +9.8	46.0 45.0	38.9 38.1
	Morning ..	217.9	42.6	23.77	0.89	20.19	+6.3	44.8	37.2
		218.8 217.2	45.1 40.86	24.21 22.90	1.16 0.75	20.54 19.47	+7.8 +4.4	45.4 43.9	38.4 36.1
April 113 to 120	Evening ..	215.1	44.7	21.49	0.86	18.5	+11.0	45.1	38.7
		215.7 214.8	45.6 43.8	21.74 21.14	0.88 0.85	18.96 18.03	+15.0 +4.8	45.3 44.8	39.3 37.9
	Morning ..	217.1	43.4	22.93	0.99	15.0	+5.1	44.7	39.4
		219.6 215.3	44.96 41.34	24.72 22.00	1.14 0.89	21.24 18.79	+8.3 -1.4	45.4 44.0	40.5 38.1



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THE MUTUAL APPLICABILITY OF THE  
ANALYTICAL FIGURES FOR BUTTER  
FAT AND GHEE

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ACADEMY OF NATURAL SCIENCES

MEMOIR

OF THE

# THE MUTUAL APPLICABILITY OF THE ANALYTICAL FIGURES FOR BUTTER FAT AND *GHEE*.\*

BY

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[ Received for publication on the 17th March, 1921. ]

A FAIR amount of analytical data has been accumulated regarding the chemical and physical properties of butter fat. In view of the increased interest being shown in maintaining the purity of foods such data are of considerable value. Butter fat, however, comes upon the market after having been subjected to one of various forms of treatment. It may have been converted into *ghee* (clarified butter) after the cream has been heated and ripened naturally or with a starter. In the process of heating the temperature of the butter fat may have been raised as high as 155°-160°C. On the other hand, in the ordinary method of preparation of butter, the cream taken from milk by a separator may have been kept constantly cold and simply churned until butter forms. The available figures dealing with the fat of the milk of Indian cows and buffaloes are not usually accompanied by a detailed account of the conditions under which the butter or *ghee* samples analysed have been prepared. It is necessary therefore to know whether the analytical figures for butter fat which has been simply taken from the milk and churned will be very different to those of the same butter fat heated, ripened and again heated to make *ghee*. This will enable a decision to be formed whether the analytical figures obtained for butter fat as butter are applicable also to butter fat as *ghee* or *vice versa*.

\* Paper read at the Eighth Indian Science Congress, Calcutta, January 1921.

In the experiments to be discussed in this paper cream was separated from cow or buffalo milk and portions of it converted into butter or *ghee* as follows :—

Cream unripened after separating and churned immediately.

The same converted into *ghee*.

Cream naturally ripened by allowing to stand overnight and then churned into butter.

The same butter converted into *ghee*.

Cream ripened with starter obtained from local butter-milk.

The same converted into *ghee*.

Cream ripened with Hansen's starter and churned.

The same converted into *ghee*.

In the manufacture of butter, the cream was kept at a low temperature as is the usual dairy practice. The *ghee* was prepared by heating the butter to 155°C. until clear. The pure butter fat required for analyses was prepared by melting the butter or *ghee* at a temperature not exceeding 60° C. and passing the melted fat through filter paper. Samples A, A.1, B, B.1, C, C.1, D, D.1 represent the fat of a herd of buffaloes treated as above.

Samples E, E.1, F, F.1, G, G.1, H, H.1 were obtained exactly as in the cases of A to D.1 except that the milk was boiled before separating the cream. This is in accordance with the practice usually followed in Indian houses.

The samples described represent butter or *ghee* prepared by all the methods prevailing in India. Milk boiled and unboiled, cream ripened by various starters or unripened, have all been included in the observations made.

Samples O to V.1 were from cows' milk treated in exactly the same way as buffalo milk samples A to H.1.

The method of preparing the various samples is tabulated below :—

Method of preparation	BUFFALO MILK SAMPLES		COW MILK SAMPLES	
	Unboiled	Boiled	Unboiled	Boiled
(1) Butter from unripened cream churned immediately .. .. .	A	E	O	S
<i>Ghee</i> from above .. .. .	A.1	E.1	O.1	S.1
(2) Butter from cream naturally ripened by allowing to stand overnight and then churned .. .. .	B	F	P	T
<i>Ghee</i> from above .. .. .	B.1	F.1	P.1	T.1
(3) Butter from cream ripened with starter obtained from local market .. .. .	C	G	Q	U
<i>Ghee</i> from above .. .. .	C.1	G.1	Q.1	U.1
(4) Butter from cream ripened with an artificial starter and churned .. .. .	D	H	R	V
<i>Ghee</i> from above .. .. .	D.1	H.1	R.1	V.1

The upper half of Table I gives the figures obtained for buffalo milk unboiled and figures in the lower half of the table are for the same milk boiled before separating off cream for butter and *ghee*-making. The butters prepared by different methods can be compared with each other as can also the various samples of *ghee*. Further the figures obtained for a *ghee* sample can be compared with the butter from which it was prepared. In no case is there any marked difference in the Reichert Meissl, saponification, butyro refractometer and other figures which are generally used for judging the purity of a sample of butter or *ghee*.

Table II gives similar figures for boiled and unboiled cows' milk and the same remarks apply as in the case of buffalo milk. The results are worthy of note in view of the fact that all *ghee* samples had been heated to a temperature of 155°C. The authors conclude therefore that the results of the analysis of *ghee* or butter can be generally applied to butter fat from Indian milch animals without reference to the method by which the butter or *ghee* was prepared. The milk from which the butter fat was obtained was drawn from the herd in the month of September, 1920, and the ration fed to the animals at the time was composed of linseed cake, cotton seed and *tur* husk with green grass as a bulky fodder.

The samples dealt with so far in this paper have been of undoubted purity and derived from the herds of milch cattle on a Government Farm. It was thought desirable, however, to examine the ordinary butter sold in the bazaar in the same way. Sample X represents good butter as generally on sale to the public, X. 1 being *ghee* prepared from the same. Samples Y and Y. 1 are of medium quality butter and the *ghee* produced therefrom. There was no guarantee that samples X and Y were from cows or buffaloes or were even pure butter. The results obtained as tabulated in Table III again confirm our previous conclusion that the pure butter fat contained in either butter or the *ghee* prepared from the latter is not materially affected by the heating to which the *ghee* is subjected. The analytical figures for the butter could be applied to *ghee* and *vice versa*.

The authors had at their disposal some samples of pure butter fat which had been kept for some years. These were also examined before and after heating to the temperature of 155°C. which is the temperature reached in the process of *ghee*-making. The figures as tabulated in Table IV again show that the heating has no significant effect on the properties of butter fat.

The authors therefore conclude that whether butter fat is kept at a temperature below 60°C. or heated to a temperature of 155°C. the analytical figures obtained in the methods usually employed in the analyses of butter fat

are not materially affected. Published analyses of butter fat on the one hand or Indian *ghee* on the other are therefore mutually applicable.

### Summary.

1. Published analyses of *ghee* and butter prepared from the milk of Indian milch animals have not been always comparable as it was not known in every case how the samples were prepared.

2. In view of the fact that butter is prepared at a low temperature and *ghee* at a high temperature, the authors thought it desirable to prepare butter and *ghee* respectively from the same sample of milk. Following the Indian custom, samples were also prepared after boiling the fresh milk.

3. The cream was subjected to one of the following methods of treatment:—

- (a) Unripened cream churned at once.
- (b) Cream allowed to stand and then churned.
- (c) Cream ripened with starter obtained from ordinary butter-milk and then churned.
- (d) Cream ripened with an artificial starter and then churned.

These methods represent the processes generally used for butter-making in India.

4. From the results obtained, it is apparent that the various methods of ripening the cream and preparing butter at a low temperature or *ghee* at a high temperature do not have any significant effect on the analytical figures which are usually taken as criteria of purity.

5. Determinations were also made on samples of butter fat, which have been kept for three to six years and on *ghee* prepared from the same. It was again found that heating the butter fat to a high temperature as is necessary in the process of *ghee*-making does not materially affect the analytical figures or the conclusions which can be drawn from them.

6. The authors therefore conclude that the analytical figures published for butter fat are applicable also to *ghee* and *vice versa*.



TABLE I.

*Butter fat from buffalo milk unboiled before separating cream.*

Sample No.	Saponi- fication No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lalle- ment	Butyro refractive index @ 40° C.	Melting point	Percentage of lactic acid in cream before churning
A. Butter	226.4	30.15	32.35	1.19	28.80	-12.2	41.9	40.8	0.11
A.1. Ghee	226.7	31.32	32.35	2.43	27.77	-12.0	41.2	42.0	
B. Butter	228.3	31.92	32.10	0.95	27.84	-9.6	41.6	40.6	0.52
B.1. Ghee	228.2	32.22	31.42	1.03	27.59	-18.1	41.8	41.8	
C. Butter	227.7	32.08	31.92	0.81	28.33	-15.6	41.9	40.0	1.22
C.1. Ghee	228.6	31.88	31.89	1.06	28.10	-15.2	41.8	42.2	
D. Butter	226.9	31.34	31.96	1.26	28.10	-9.1	41.9	40.8	0.57
D.1. Ghee	228.3	31.27	31.76	1.16	27.86	-7.4	41.7	42.2	

*Butter fat from buffalo milk boiled before separating cream.*

E. Butter	227.2	31.50	32.06	0.89	27.93	-9.3	41.9	41.0	0.11
E.1. Ghee	226.5	31.94	37.76	1.31	27.29	-7.6	41.9	41.8	
F. Butter	227.8	32.12	31.82	0.85	27.78	-6.1	41.9	41.0	0.44
F.1. Ghee	226.7	31.63	31.33	1.23	27.32	-9.9	41.7	41.6	
G. Butter	225.5	31.97	31.59	0.84	27.29	-12.9	41.6	41.0	1.90
G.1. Ghee	225.3	31.85	31.19	1.12	26.88	-14.8	41.8	42.0	
H. Butter	226.8	31.97	31.79	0.83	26.86	-11.0	41.7	40.6	1.12
H.1. Ghee	226.5	31.38	31.82	0.93	27.01	-4.7	41.8	42.0	

TABLE II.

*Butter fat from cow milk unboiled before separating cream.*

Sample No.	Saponi- fication No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lalle- ment	Butyro refractive index @ 40° C.	Melting point	Percentage of lactic acid in cream before churning
O. Butter	223.9	38.57	24.70	1.44	19.60	-1.0	43.8	35.6	0.11
O.1. Ghee	222.6	37.54	24.58	3.05	19.17	+0.4	43.8	36.0	
P. Butter	222.1	38.00	24.60	1.43	19.80	+1.0	43.9	36.4	0.54
P.1. Ghee	221.5	38.54	24.34	1.77	19.33	+0.9	43.5	36.6	
Q. Butter	221.9	37.63	24.37	0.94	19.33	+0.6	43.2	36.4	1.11
Q.1. Ghee	221.2	37.68	24.70	3.56	19.94	+7.1	43.6	37.2	
R. Butter	221.7	37.66	24.66	1.48	18.81	+4.6	43.8	36.4	0.58
R.1. Ghee	221.2	37.61	24.94	3.02	19.74	+6.2	43.5	36.6	

*Butter fat from cow milk boiled before separating cream.*

S. Butter	222.4	38.02	24.48	1.30	19.08	+7.0	43.6	36.6	0.11
S.1. Ghee	222.0	38.22	24.48	1.58	18.73	+11.2	43.5	38.6	
T. Butter	222.0	37.38	24.70	1.40	19.63	+4.7	43.7	37.4	0.33
T.1. Ghee	221.8	38.67	24.53	1.38	19.85	+4.8	43.7	37.4	
U. Butter	222.8	38.94	24.57	1.40	19.45	-1.1	43.0	37.0	
U.1. Ghee	220.8	37.54	24.56	2.84	21.29	+7.0	43.4	38.0	
V. Butter	222.0	37.96	24.38	1.83	18.91	+5.4	43.6	37.4	
V.1. Ghee	222.6	38.07	24.38	2.46	19.06	+3.1	43.5	37.8	



TABLE III.

Sample No.	Saponi- fication No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lalle- ment	Butyro refrac- tive index @ 40° C.	Melting point
X. Good butter	227.2	26.3±	31.77	1.13	25.97	-14.6	41.0	40.0
X.1. <i>Ghee</i> from above ..	226.3	26.7±	31.38	1.52	25.80	-15.3	40.6	40.0
Y. Medium quality butter ..	214.1	35.90	19.97	0.60	18.23	+15.3	44.0	43.0
Y.1. <i>Ghee</i> from above ..	214.5	35.81	19.84	0.53	18.42	+12.3	43.9	43.8

TABLE IV.

Sample No.	Saponi- fication No.	Iodine Wijs' No.	Reichert Meissl No.	Polenske No.	Kirschner No.	Ave Lalle- ment	Butyro refrac- tive index @ 40° C.	Melting point	Age of sample
I Butter from individual buffalo ..	240.1	21.89	32.52	1.91	26.13	-126.8	42.5	38.0	6 years
I.1. <i>Ghee</i> from above ..	238.0	21.54	32.72	2.22	26.62	- 71.6	42.6	38.2	..
J Butter from individual cow ..	229.8	25.97	24.52	1.49	20.14	- 51.0	44.7	40.2	4 years
J.1. <i>Ghee</i> from above ..	229.8	26.60	24.34	1.91	20.10	- 41.0	44.2	40.2	..
K Butter from herd of buffaloes.	230.0	29.18	22.89	1.05	19.31	- 64.2	45.9	42.4	4 years
K.1. <i>Ghee</i> from above ..	228.7	28.98	22.06	0.83	18.96	- 57.6	45.8	42.6	..
L Butter from herd of cows ..	225.3	31.84	21.45	1.68	16.15	- 53.0	45.9	38.8	4 years
L.1. <i>Ghee</i> from above ..	225.4	32.12	21.15	2.26	16.72	- 48.4	46.0	39.0	..
M Butter from herd of buffaloes.	219.8	37.50	22.24	0.95	19.56	- 31.2	46.5	40.0	3½ years
M.1. <i>Ghee</i> from above ..	218.4	37.45	22.02	1.84	19.47	- 30.1	45.3	39.8	..
N Butter from herd of cows ..	220.0	33.69	18.98	1.47	15.04	- 30.0	45.3	40.0	4½ years
N.1. <i>Ghee</i> from above ..	221.2	33.37	18.76	1.74	15.21	- 35.1	46.3	39.6	..

# INVESTIGATIONS ON INDIAN OPIUM NO. 3.

## STUDIES IN THE MECONIC ACID CONTENT OF INDIAN OPIUM.

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CERTAIN organic acids such as malic, citric, succinic, oxalic and tannic acids appear to be of common occurrence in many species of plants. Quinic acid occurs in the bark of cinchonas and in coffee roots and has recently been found by Tanret<sup>1</sup> in *Cedrus Libani*. Lactic acid has been reported to occur in the sap of the vine<sup>2</sup> and in opium.<sup>3</sup> Fluckiger and Hanbury,<sup>4</sup> however, state that it is probably not an original constituent of poppy juice. We agree with this view and also suggest that lactic acid in the sap of the vine has been produced by fermentation having no connection with the life processes of the plant. Meconic acid has so far only been met with in opium, the produce of the opium poppy. Since meconic acid is strictly peculiar to opium it would appear that a study of the amount of this acid in opium samples produced under different experimental conditions might lead to an increased knowledge of the physiology of the opium poppy plant.

### METHOD OF ANALYSIS.

The methods available appeared to us unsatisfactory and we adopted the following method of estimating meconic acid in opium. It is being fully described in the *Analyst*.

<sup>1</sup> *Comptes rend.*, 1921, **172**, 234.

<sup>2</sup> Thorpe. *Dict. App. Chem.*, III, p. 221.

<sup>3</sup> T. & H. Smith. *Pharm. Jour.* (ii), **7**, 50.

<sup>4</sup> *Pharmacographia*.

Five gramm. of opium are rubbed up carefully with water in a mortar and transferred to a stoppered flask using 50 c.c. of water in all. The flask is well shaken and allowed to stand overnight. The liquid is then filtered and a measured quantity, *e.g.*, 40 or 45 c.c. of the filtrate, is taken for meconic acid estimation.

Six c.c. of 50 per cent.  $\text{CaCl}_2$  solution are added, the liquid shaken and allowed to stand 24 hours. Any attempt to partially neutralize the opium solution before addition of the  $\text{CaCl}_2$  always results in the production of meconic acid contaminated with colouring matters. The precipitate is then filtered off in a Hirsch funnel and washed with water till the washings are colourless and consists almost entirely of calcium meconate and calcium sulphate. It is almost perfectly white. The precipitate is transferred to a small beaker by the aid of 15 c.c. of 1.25 N hydrochloric acid, the filter paper being removed. The beaker is then heated on the water bath until the precipitate has completely dissolved. The liquid is then allowed to stand in a cool place for 24 hours. Meconic acid separates in white crystalline scales in highly pure condition. It is filtered in a Hirsch funnel, being removed completely from the beaker by means of the mother liquor and then washed twice with  $\frac{1}{2}$  c.c. distilled water, dried between filter paper and then in a dessicator over  $\text{H}_2\text{SO}_4$  for 3 hours and weighed as  $\text{C}_7\text{H}_4\text{O}_7 \cdot 3\text{H}_2\text{O}$ .

The amounts of water used for extraction of the opium and of hydrochloric acid used for decomposition of the calcium meconate precipitate were decided on after a series of experiments with pure meconic acid.

The result is then calculated by adding 0.0213 gramm. to the weight of meconic acid found to correct for the solubility of meconic acid in 15 c.c. of 1.25 N HCl. We then multiply the figure obtained by a factor depending on the amount of the aliquot portion of the opium solution taken for analysis. If this were 45 c.c. we multiply by 50/45. We multiply the figure obtained by 10/9, since we have found that roughly about 10 per cent. of the meconic acid is unprecipitated by the calcium chloride. The following example illustrates the method of calculation. In a certain analysis we took 5 gramm. of opium and extracted with 50 c.c. water. We took 44 c.c. of the filtrate for precipitation with  $\text{CaCl}_2$  and obtained 0.2744 gramm. meconic acid. The calculation is as follows:—

$(0.2744 + 0.0213) \times \frac{50}{44} \times \frac{10}{9}$  = corrected weight of meconic acid from 50 c.c. of solution or 5 gramm. opium.

$\therefore$  in 100 gramm. opium we get  $(0.2744 + 0.0213) \times \frac{50}{44} \times \frac{10}{9} \times \frac{100}{5}$   
= 7.47 per cent. meconic acid in opium.

## THE MECONIC ACID CONTENT OF OPIUM.

In our previous memoirs<sup>1</sup> in this series we have examined the effect of various factors both environmental and non-environmental on the morphine content of Indian Opium. The experimental samples made use of for that work were available for the work described in the present paper. There was, however, no need for a complete investigation of the meconic acid content of these samples, for it was soon discovered that the meconic acid content varies almost directly with the total alkaloid content of the opium. Factors modifying the alkaloidal content therefore necessarily modify meconic acid extent.

Our investigations on the alkaloidal content of opium have established the fact that when an opium capsule is lanced for opium on a number of successive occasions at one or more days' intervals the morphine content of the opium rapidly diminishes in the product of each succeeding lancing.<sup>2</sup>

We find also that the meconic acid content of the opium of a series of successive lancings of a capsule falls off in a manner somewhat similar to the way in which the morphine content diminishes. The following table sets out the results of the analyses of the opium of a number of successive lancings of poppy capsules as regards the morphine and meconic acid content.

*Composition of opium in percentage on dry matter.*

Series No.					No. OF LANCING			
					1st	2nd	3rd	4th
I	(a) morphine	..	..		13.3	9.5	6.0	4.3
	(b) meconic acid	..	..		9.7	7.0	5.8	5.8
II	(a) morphine	..	..		13.3	10.2	5.0	1.2
	(b) meconic acid	..	..		9.6	8.0	5.0	3.7
III	(a) morphine	..	..		13.8	11.4	7.9	..
	(b) meconic acid	..	..		9.7	8.1	5.6	..
IV	(a) morphine	..	..		13.6	9.2	5.9	..
	(b) meconic acid	..	..		8.1	6.1	4.2	

It will be seen that in the first three lancings at least there is a fairly rapid successive diminution in meconic acid content. The fall is not so marked as it is in the case of morphine, yet it is distinct.

Opium of second lancings always contains less meconic acid than opium of first lancings and the third lancings contain less still. In the fourth

<sup>1</sup> *Mem. of the Dept. of Agri. in India*, Vol. VI, Nos. 1 and 2.

<sup>2</sup> *Mem. of the Dept. of Agri. in India*, Vol. VI, No. 1.



lancings, however, the meconic acid content does not appear notably less than in the third lancings.

A number of other samples of opium taken from our collection containing widely varying percentages of morphine were next submitted to analysis for meconic acid content. The following results were obtained, the figures being arranged in order of the highest morphine content opium downwards.

*Composition of dry matter of opium.*

No. of sample	Morphine per cent.	Meconic acid per cent.
372	18.44	9.79
373	18.14	9.90
1077-87	17.00	9.17
43	16.35	10.55
49	15.97	9.29
55	15.25	9.43
61	15.22	9.70
108	14.18	7.08
37	14.12	9.58
67	14.11	9.40
44 (1)	13.80	9.72
270 (1)	13.62	8.06
19	13.51	8.50
144	13.29	9.57
386	13.26	9.73
25	13.18	8.53
31	13.04	8.47
44 (2)	11.40	8.06
147	10.20	7.99
387	9.50	7.02
270 (2)	9.16	6.12
44 (3)	7.90	5.56
388	6.01	5.77
270 (3)	5.92	4.25
148	5.05	4.99
389	4.35	5.83
620 H	1.87	3.56
596 H	1.74	4.04
150	1.18	3.75
Hill sample	0.00	4.67

The figures show points of some interest. In the first place opiums of high morphine content always contain a high percentage of meconic acid. Roughly speaking opiums containing 16 per cent. morphine contain 10 per cent. meconic acid. We have examined opiums containing less than 2 per cent. morphine but these still contain 3 to 4 per cent. meconic acid. A sample of opium showing no morphine by the British Pharmacopœia process yet still showed 4.67 per cent. meconic acid. In the case of high morphine content opiums, however, we never find a low content of meconic acid. Thus an

opium containing 13 per cent. morphine is always found to contain at least 8.5 per cent. meconic acid.\*

The foregoing results thus showed that though samples of opium poor in morphine also contained small amounts of meconic acid and vice versa yet the relation between morphine and meconic acid content was not direct. It was accordingly resolved to make a more complete analysis of a series of opium samples. Among our collections we have many sets, each representing the opium of successive lancings of the same capsules. We selected four of these sets at random, each set consisting of three successive lancings of the same capsules. Thus there were 12 samples in all. Each sample was analysed for morphine, codeine, narcotine and papaverine, meconic acid and soluble sulphate. For the morphine estimations we made use of the method of the British Pharmacopœia (1914). In the case of codeine we used our own method<sup>1</sup> or rather a slight improvement of it which is shortly to be published. For narcotine and papaverine we used a new method which we are communicating to the *Analyst*. This latter method is very accurate and estimates narcotine and papaverine together.

Our results are summarized in the following table.

Sample No.	Description of sample			PERCENTAGE ON DRY MATTER OF OPIUM						
				Morphine	Codeine	Narcotine + Papaverine	Total alkaloids	Water soluble sul- phate as SO <sub>4</sub>	Meconic acid	Calculated meconic acid required for alkaloids estimated
170	1st lancings	1917-18	..	11.54	2.85	3.81	23.20	2.40	9.41	9.09
171	2nd do.	1917-18	..	7.47	2.75	7.81	18.03	2.87	6.90	6.92
172	3rd do.	1917-18	..	4.04	2.29	6.90	13.23	3.26	5.32	4.92
320	1st lancings	1918-19	..	12.07	3.05	11.77	26.89	2.08	11.10	10.37
322	2nd do.	1918-19	..	9.84	3.06	11.53	24.43	2.56	9.54	9.30
324	3rd do.	1918-19	..	6.11	3.64	9.71	19.46	3.43	7.35	7.30
301	1st lancings	1919-20	..	12.09	3.29	12.00	27.38	2.38	11.07	10.63
302	2nd do.	1919-20	..	8.80	3.27	10.03	22.15	2.96	8.01	8.54
303	3rd do.	1919-20	..	4.68	3.16	8.60	16.44	3.35	6.34	6.17
386	1st lancings	1920-21	..	13.10	3.21	10.90	27.21	2.62	10.63	10.61
387	2nd do.	1920-21	..	9.69	2.92	8.07	20.68	3.28	8.10	8.09
388	3rd do.	1920-21	..	6.76	2.81	6.90	16.47	3.97	6.88	6.36

\* We found one exception, viz., No. 108, which showed only 7.08 per cent. meconic acid whereas it contained 14.18 per cent. morphine.

<sup>1</sup> *The Analyst*, September 1920.



A column has been added to the table showing the amount of meconic acid theoretically required to combine with the morphine, codeine, narcotine and papaverine actually found. Two molecules of the alkaloid require one molecule of  $C_7H_4O_7 \cdot 3H_2O$ .

The table brings out the striking fact that the meconic acid content of opium is almost exactly what would be required to ensure that the alkaloids are present solely as meconates. Thus the amount of meconic acid found agrees fairly closely in practically every case with the amount calculated as being necessary to convert all the alkaloids to meconates. As a rule the actual amount of meconic acid found appears to be slightly greater than the calculated amount. In this connection it must be remembered that there are a number of other rarer alkaloids present in opium whose total amount is about 1 per cent. and which would require roughly 0.25 per cent. of meconic acid to enable them to exist as meconates. Any slight irregularity between the actual meconic acid found and the calculated amounts might easily be due to inaccuracies in analytical methods. Thus we do not claim absolute accuracy for our method of estimating meconic acid and the British Pharmacopœia method of estimating morphine is after all only approximate.

The agreement in so many cases between the actual amounts of meconic acid found and the amounts calculated as being necessary for the alkaloids to be present as meconates is so remarkable that one must be forced to the conclusion that the alkaloids occur in the latex solely as meconates. The acid reaction of opium is easily accounted for owing to the weakly basic nature of narcotine and papaverine, the meconic acid equivalent to them reacting as free acid. This relationship between the total alkaloid content and the meconic acid content of the latex has one interesting bearing on our former work. We have shown that the morphine content diminishes in the latex of each successive lancing. We have been unable to find that the decrease in morphine content is accompanied by any increase in the content of any other alkaloid. The relationship above referred to is confirmatory evidence that the decrease in morphine content of the lancing is not counterbalanced by an increase in any other alkaloid so far not estimated.

The above table also shows that the sulphate content increases in each successive lancing and thus bears almost an inverse relationship to the alkaloids content. It is unlikely, therefore, that the alkaloids occur as sulphate.

These facts are interesting as showing the form in which the alkaloids occur in the latex. Dott<sup>1</sup> states that morphine occurs in opium half combined as

<sup>1</sup>Watt's *Dict. of Chem.*, III, 437.

meconate and half as sulphate but the foregoing results show that this is not the case.

It will be convenient to refer here to the fact that the total ash constituents of the latex vary inversely with the total alkaloid content. Thus the total ash content of each successive lancing increases whereas the alkaloidal content rapidly diminishes. The ash constituents of opium will, however, form the subject of a separate memoir in this series.

#### CONCLUSIONS.

1. The meconic acid content of opium varies directly as the total alkaloid content and the acid appears to be present in approximately sufficient amounts to enable all the alkaloids to be combined as meconates. It would thus seem that the physiological process whereby alkaloids are produced in the opium poppy produces meconic acid in equivalent amount to the alkaloids.

2. The soluble sulphate content of the latex increases as the alkaloidal content diminishes. From the table on p. 219, it can be calculated that in the early lancements there is insufficient sulphate to completely combine with the total alkaloids but in the later lancements there is more than sufficient for this purpose.

It appears most likely, however, that the sulphate is present in a mineral form and that the alkaloids are present as meconates only, the acid reaction of opium being due to the dissociation of the meconates of narcotine and papaverine, which alkaloids are so weakly basic.



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# CHEMICAL STUDIES ON SAFFLOWER SEED AND ITS GERMINATION.

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## I. HISTORICAL.

THE germination of oil seeds has been often studied, but it cannot be said that the process can be, even yet, completely followed. The process by which the oil is converted into materials directly capable of being utilized by the seedling seems to be a very complicated one, largely dependent on the action of several enzymes. Most of the investigations made hitherto have been done on castor seed, a few on groundnut, but no worker has hitherto used the safflower seed (*Carthamus tinctorius*). This seed, however, has particular interest in that the oil in this seed is a very oxidisable one, and the phenomena connected with its germination may offer peculiar and interesting features. The seed, moreover, germinates very easily and very quickly, and is in every way suited for an investigation of this character.

There is no need to recall the earlier investigations on the germination of oil seeds. A full account of these can be found in the papers by Green and Deleano referred to below. The position in 1890, chiefly, be it said again, as a result of investigation on castor seed, was stated by Green<sup>1</sup> as follows:—

1. "The reserve materials in the endosperm of *Ricinus communis* consist chiefly of oil and proteid matters, the latter being a mixture of globulin and albumose.

2. "The changes during germination are partly due to enzyme action, there being three enzymes present in the germinating seed. One is a protease resembling trypsin, the second splits oil into fatty acid and glycerine, the third is a rennet enzyme.

<sup>1</sup> Green. *Proc. Roy. Soc.*, vol. 48 (1890), page 370.



3. "At least two of these and therefore presumably all of them are in a zymogen condition in the resting seed, and become active in consequence of the metabolic activity set up in the cells by the condition leading to germination, especially moisture and warmth.

4. "The changes caused by the enzymes are followed by others, due to the metabolism of the cells, these being processes of oxidation.

5. "The embryo exercises some influence on the latter, setting up as it develops a stimulus probably of a physiological description.

6. "The result of the various processes is to bring about the following decompositions :—

The proteids are, by the enzyme, converted into peptone and later into asparagin.

The oil is split by the glycerid enzyme into fatty acid and glycerine, the latter gives rise to sugar and the former to a vegetable acid which is soluble in water and in ether, is crystalline and has the power of dialysis.

7. "The absorption in all cases takes place by dialysis.

8. "The appearance of starch and of oil in the embryo or the young plant is due to a secondary formation and not to a translocation of either."

Further investigations by Green<sup>1</sup> added little to these results but, still working with castor seed, Deleano<sup>2</sup> has made a distinct advance, for he found and identified not only the enzymes detected by Green, but also a catalase, a peroxidase, and a reductase. It is probable that to one of these is due the oxidation noticed by Green (see para. 4 above) which he attributed to the direct effect of the cell metabolism.

The present paper attempts to determine how far some of these results are applicable to the safflower seed, the oil of which is of a very different type from that of castor and the matter, so far as it has been carried, will be discussed under the following headings :—

1. The composition of safflower seed in the resting condition.
2. The changes in the composition during germination.
3. The lipase of germinating safflower seed.
4. The oxidase of germinating safflower seed.
5. Summary and conclusions.

## II. THE COMPOSITION OF SAFFLOWER SEED IN THE RESTING CONDITION.

The investigations, of which an account follows, were conducted with seed grown near Poona and at Bijapur in the Deccan, both considerable

<sup>1</sup> Green. *Proc. Roy. Soc.*, vol. 77 B (1905), page 85.

<sup>2</sup> Deleano. *Centr. Bakt.* (2). vol. 24 (1909), page 130.

centres for the growth of the crop. Good, well-formed and ripe seed only was selected for the experiments. The seed so selected derived from both the above centres, weighed on an average 0.05 gramme per seed, the individual seed varying little from this average. Its specific gravity is greater than water, and it just floated in 4 per cent. salt (NaCl) solution. As a rule, seed of this quality formed from half to two-thirds of good commercial samples.

After removal of the husk which forms approximately fifty per cent. of the seed, and which was not further investigated, the composition of the seed kernels is shown by the following figures calculated on the completely dried materials :—

				Poona seed per cent.	Bijapur seed per cent.
Ether extract	..	..	..	58.1	56.6
*Proteids	..	..	..	31.4	29.0
Woody fibre	..	..	..	0.7	1.5
Nitrogen-free extract	..	..	..	6.7	9.9
Ash	..	..	..	3.1	3.0
				100.0	100.0
*Containing nitrogen	..	..	..	5.0	4.6

The nitrogen-free extract was further investigated as regards the presence and quantity of starch, sugars, tannin and other constituents of possible importance.

*Starch.* A preliminary test with iodine indicated that, in ripe well-formed seed, starch is totally absent, and this was confirmed by careful microscopic examination. In thin sections, iodine gave no blue coloration with any element of the seed, while aleurone grains (stained brown with iodine) were very abundant.

*Sugars.* After extraction of the crushed seed with ether the residue, on treatment with water, gave a solution which did not reduce Fehling's solution. After inversion, however, with hydrochloric acid Fehling's solution was slowly reduced.

The source of the reducing power of the inverted solution was indicated by the following experiments: Five grammes of seed kernels were extracted with boiling water for ten minutes and cooled, the solution precipitated with basic lead acetate and the lead removed with sodium phosphate. The solution again gave no reaction with Fehling's solution. On inversion, however,

reduction was obtained equivalent to 0.37 per cent. of cane sugar on the original weight of seed. The sugar thus produced gave an osazone on treatment with phenyl hydrazine, crystallizing in a form exactly similar to that given by glucosazone when slowly cooled, but which melted at  $185^{\circ}$  to  $187^{\circ}\text{C}$ . On careful recrystallization from a mixture of pyridine and alcohol, large crystals were obtained, which melted after sudden heating at  $190^{\circ}\text{C}$ . A solution of this sugar rotating  $+1.5^{\circ}$  before inversion showed a rotation of  $-1.0^{\circ}$  after inversion with acid.

The nature of the sugar is thus not very clear, as the melting point of glucosazone should be over  $205^{\circ}\text{C}$ . The matter requires further investigation, but, in the meantime, it is clear that the safflower seed in the resting condition shows no reducing sugar but contains 0.37 per cent. (calculated as cane sugar) of a sugar which is non-reducing and is decomposed by acids with inversion of the rotation.

*Glucosides.* Careful tests were made for the presence of glucosides in the seed. Fifteen grammes of crushed safflower seed kernels were extracted with ether, and then steeped in absolute alcohol for twenty-four hours. The alcoholic extract was then evaporated, and the residue tested for glucosides with ammonium molybdate. No indication of glucosides was found, and the absence of any substances of this nature soluble in absolute alcohol is thus indicated.

A similar negative result was obtained by extracting crushed seed kernels with water, and precipitating the extract with basic lead acetate. On treating the precipitate with sulphuretted hydrogen a solution was obtained which again gave no tests for glucosides. Thus no glucoside soluble in water is present.

It appears, therefore, that glucosides soluble either in water or in absolute alcohol are not present in safflower seed in the resting condition.

*Tannin.* As the presence of small quantities of tannin would have seriously interfered with the later experiments on the germinating seed, it was essential to ascertain whether this substance was present in any noticeable proportion. Fifteen grammes of crushed kernels were therefore treated with ether, and the residue extracted with hot water. The solution was precipitated with lead acetate, the precipitate decomposed with sulphuretted hydrogen, and the resulting solution concentrated to a small volume. This solution now gave only a brown tinge with ferric chloride, no coloration with ferrous sulphate and no colour with ammoniacal solution of potassium ferricyanide. There is thus no tannin present in the resting stage of safflower seed.

In general, therefore, healthy, well-formed kernels of safflower seed consist of (1) fifty-five to sixty per cent. of ether extract, (2) about thirty per cent. of proteid, (3) about one per cent. of fibrous matter unattacked by dilute acid and alkali, and (4) from six to ten per cent. of nitrogen-free extract containing no starch, no glucosides, no tannin, no reducing sugar, and a small quantity of a non-reducing sugar resembling cane sugar, but whose character is not yet made out with certainty.

### III. THE CHANGES IN COMPOSITION DURING GERMINATION.

Safflower seed germinates with great ease. The process was conducted in the first place in soil and the composition of the whole plant, including the seed kernel itself, determined at different stages.

The following stages were taken for examination in the first instance :—

- (1) Resting seed.
- (2) Seed testa just cracking.
- (3) Radicle protruding.
- (4) Lateral roots just appearing.
- (5) Root system established.

When the stage desired was reached, the seeds were removed, washed to remove adhering soil particles, dried as far as possible with paper, and then completely dried in a vacuum desiccator.

The result of the analysis of the dried material at each stage is as follows:—

		Stage (1) Per cent.	Stage (2) Per cent.	Stage (3) Per cent.	Stage (4) Per cent.	Stage (5) Per cent.
Ether extract	..	58.1	57.5	56.0	10.1	5.3
*Proteids..	..	31.4	31.6	33.3	31.6	34.1
Woody fibre	..	0.7	0.4	0.4	6.2	7.0
Nitrogen-free extract	..	6.7	7.7	7.5	41.9	40.1
†Ash	..	3.1	2.8	2.8	10.2	13.5
		100.0	100.0	100.0	100.0	100.0
*Containing nitrogen	..	5.0	5.1	5.3	5.1	5.4
†Containing	{ Sand ..	0.3	0.04	0.04	0.6	0.6
	{ Lime ..	trace	trace	trace	0.2	0.5
	{ Phosphoric acid ..	1.5	1.5	1.5	1.5	1.6
	{ Potash ..	1.4	1.4	1.4	4.1	5.7

The "ether extract" in stages (4) and (5) contained chlorophyll, and in obtaining the above figures this was removed by means of ninety-six per cent. alcohol from the original extract. The method is not however satisfactory and the figures for ether extract are doubtful in these cases, and in following tables the total crude extract only is recorded.

The above table shows that comparatively little change in general composition, as indicated by these analyses, takes place until the radicle has protruded, and the lateral roots are just forming. This is the point too, it appears, when mineral matter begins to be absorbed, for there is a considerable increase in the amount of lime and particularly in the amount of potash in the plant. To find the exact point at which the large change in composition (particularly in the reduction in the amount of oil and increase in the quantity of nitrogen-free extract) occurs, another stage was introduced in the next series of analyses between (3) and (4), while stage (2) (seed testa just cracking) was omitted.

Four pots were now sown with safflower seed in *pure sand*, distilled water being used for watering, and the seeds were removed and examined as before at the following stages :--

- (1) Resting seed.
- (2) Radicle protruding.
- (3) Radicle one inch long.
- (4) Lateral roots just spreading.
- (5) Root system established.

The dried material at each stage gave the following figures on analysis :—

	(1) Per cent.	(2) Per cent.	(3) Per cent.	(4) Per cent.	(5) Per cent.
Ether extract .. ..	56.6	54.5	44.8	30.0	17.7
*Proteids .. ..	29.0	27.6	27.4	26.0	25.4
Woody fibre .. ..	1.5	1.7	2.0	3.1	5.3
Nitrogen-free extract .. ..	9.9	13.4	22.6	37.6	47.9
†Ash .. ..	3.0	2.8	3.2	3.3	3.7
	100.0	100.0	100.0	100.0	100.0
* Containing nitrogen.. ..	4.6	4.4	4.4	4.2	4.1
{ Sand .. ..	0.6	0.1	0.3	0.4	0.7
{ Lime .. ..	trace	trace	trace	trace	trace
† Containing { Phosphoric acid .. ..	1.4	1.4	1.4	1.3	1.3
{ Potash .. ..	1.3	1.3	1.3	1.4	1.5



These figures indicate a much more regular and gradual reduction in the amount of ether extract than appeared in the previous ones and an almost corresponding increase in the amount of nitrogen-free extract and fibre taken together.

The changes in the amount and character of the sugars at these stages are interesting and are given in the following table :—

	(1) Per cent.	(2) Per cent.	(3) Per cent.	(4) Per cent.	(5) Per cent.
Total sugar .. ..	0.37	0.37	0.52	0.84	1.22
Reducing sugar (calculated as glucose) .. ..	nil	trace	0.33	0.29	0.21
Non-reducing sugar (calculated as cane sugar) .. ..	0.37	0.37	0.19	0.55	0.94

There is thus a general rise in the total amount of the sugars till germination is completed and the plant is capable of supplying its food from outside; the reducing sugars on the other hand increase to an amount of 0.33 per cent. followed by steady reduction to 0.21 per cent., while the non-reducing sugar after a slight decrease in stage (3) rises rapidly to the end of the process.

Starch could not be detected at any stage of the germination.

The germination process is also characterized by a gradual increase in the solubility of the proteid matter, as is indicated in the following table :—

	(1) Per cent.	(2) Per cent.	(3) Per cent.	(4) Per cent.	(5) Per cent.
Total proteids .. ..	29.0	27.6	27.4	26.0	25.4
Insoluble proteids .. ..	27.9	26.3	23.7	15.2	7.9
Soluble proteids .. ..	1.1	1.3	3.7	10.8	17.4

There is thus a slight but regular and gradual fall in the total proteid matter. After stage (3) when the radicle is fully formed and before



lateral roots commence to develop, there is a sudden large increase in the soluble proteids which continues until germination is completed.

The characteristic features of germination of a highly oily seed like that of safflower are therefore (1) a gradual decrease in the oil content, (2) a gradual increase in the nitrogen-free extract (consisting largely of carbohydrates) which is very marked in the case of the sugars, (3) a gradual but slight loss of proteids, but a large increase in the proportion of soluble proteids. The changes indicated commence from the beginning, but become rapid after the radicle is well grown and just before lateral roots begin to be formed, and they all continue until the process is complete.

#### IV. THE LIPASE OF GERMINATING SAFFLOWER SEED.

In the reactions taking place during the germination of all oily seeds hitherto examined, a lipase takes a considerable part. To ascertain whether this was also the case with safflower seed, one hundred grammes of seed was allowed to germinate on a sterilized wet cloth. After the radicle had grown an inch long (two to three days) the whole was crushed and extracted with 200 c.c. of 5 per cent. sodium chloride solution containing preservative, and filtered through cloth. This extract was then added to safflower oil kept at a constant temperature of 35°C. and the acidity produced determined from day to day, using a similar sample of extract which had been boiled as a control.<sup>1</sup>

In doing this great difficulty was experienced in preventing the growth of mould in the testa. The presence of 0.2 per cent. potassium cyanide solution, which was at first employed, seemed quite insufficient for this purpose, for in every case mould was visible to the naked eye after from seven to eleven days, and mycelia could be detected earlier on microscopic examination. Before, therefore, an experiment on the lines above indicated could give satisfactory results, an investigation had to be made as to the most suitable preservatives for preventing the growth of mould in connection with safflower seed and safflower seed extract.

Seed was therefore sown on sterilized blotting paper after being steeped in various strengths of copper sulphate solution, in 0.1 per cent. formalin solution, and in water saturated with chloroform. The results were as follows :—

- (1) Seed steeped in  $\text{CuSO}_4$  solution (0.5 per cent.) for 15 minutes. Mould appeared in three days.

<sup>1</sup> This method was essentially used by Green in studying the enzymes of castor seed. *Proc. Roy. Soc.*, vol. 48 (1890), p. 370.

- (2) Seed steeped in  $\text{CuSO}_4$  solution (1.0 per cent.) for 15 to 20 minutes. Mould appeared, but less vigorously.
- (3) Seed steeped in  $\text{CuSO}_4$  solution (2.0 per cent.) for 20 minutes. Mould appeared but only to a slight extent.
- (4) Seed steeped in 0.1 per cent. solution of formalin for 10 to 15 minutes. Mould appeared in four days.
- (5) Seed steeped in water saturated with chloroform for half an hour. Mould appeared in two to three days.

This is perhaps not unexpected, for the vitality of mould spores on seeds has been proved to be very great, even in presence of copper sulphate. The safflower seed<sup>1</sup>, moreover, is well adapted to preserve them from injury by the steeping solution employed.

The secret, however, of the development of moulds was found to be germination on paper. If the use of paper was abandoned, and seeds previously treated with 2 per cent. copper sulphate solution were germinated in sterilized sand, no difficulty was ever found with the appearance of mould during germination, and no trace of its presence was ever detected by microscopic or other tests.

Apart entirely from the question of the development of moulds during germination, the method outlined above of extracting the enzyme was found to be unsatisfactory. It had been used by Green<sup>2</sup> in his investigations on the germination of castor seed, but it was abandoned in the present case because the potassium cyanide used for preventing the growth of moulds decomposed on standing in the dilute solution used, giving alkali which rendered doubtful any determinations of acidity.

Instead of this, the following method originally employed by Armstrong<sup>3</sup> was used. The seed, germinated to the extent desired, was dried and extracted with ether at the ordinary temperature. The residue was freed from ether by standing in the air, and the dried and powdered material which remained was tested for enzyme by allowing it to act on an emulsion of the oil with a little gum arabic solution. The extent of the acidity developed in the oil was used as a measure of the amount of lipase.

In the first series of experiments made to test this method with safflower seed in course of germination the following figures were obtained. Chloroform was added to the liquid every day, but no other sterilization was carried on. The acidity is represented by the number of cubic centimeters of

<sup>1</sup> Lesage. *Bull. Soc. Scientifique de l'Ouest*, vol. 21, p. 129 (Rennes 1912).

<sup>2</sup> Green. *Proc. Roy. Soc.*, vol. 48 (1890), p. 370.

<sup>3</sup> Armstrong. *Proc. Roy. Soc.*, vol. 78 (1906), p. 376.

centinormal potassium hydroxide required for neutralization, using phenolphthalein as indicator.

	(1)	(2)	(3)	(4)	(5)
	Control 10 c.c. oil only c.c.	10 c.c. oil, 0.5 gm. powder from seed c.c.	10 c.c. oil, 1.0 gm. powder from seed c.c.	10 c.c. oil, 1.5 gm. powder from seed c.c.	10 c.c. oil, 2.0 gm. powder from seed c.c.
Original acidity .. ..	2.0	7.5	13.0	15.7	19.8
Additional acidity—					
After first day .. ..	0.6	3.8	9.7	20.6	25.9
„ second day .. ..	0.4	9.2	14.3	28.6	31.7
„ third day .. ..	0.4	8.7	18.2	20.3	29.8
„ fourth day .. ..	0.3	8.5	21.5	16.2	25.7
„ fifth day .. ..	0.3	9.2	23.4	4.7	20.6
„ sixth day .. ..	0.2	3.0	26.1	3.6	6.9
„ seventh day .. ..	0.15	0.8	8.1	2.6	5.1
„ eighth day .. ..	nil	0.5	3.3	1.3	3.6
„ ninth day .. ..	nil	0.4	2.5	0.2	1.8
„ tenth day .. ..	nil	nil	1.2	nil	nil
„ eleventh day .. ..	nil	nil	nil	nil	nil
TOTAL ADDITIONAL ACIDITY ..	0.37	44.1	128.3	98.1	151.1

If we subtract the original acidity in each case and also the acidity of the control on each day, we have for the first five days as follows:—

	(2) 0.5 gm. powder c.c.	(3) 1.0 gm. powder c.c.	(4) 1.5 gm. powder c.c.	(5) 2.0 gm. powder c.c.
Total acidity—				
After one day .. ..	3.2	9.1	20.0	25.3
„ two days .. ..	12.0	23.0	48.2	56.6
„ three „ .. ..	20.3	40.8	68.1	86.0
„ four „ .. ..	28.5	62.0	84.0	111.4
„ five „ .. ..	37.4	85.1	88.4	131.7

From the second to the fourth day the amount of acidity produced is thus very closely proportional to the amount of seed powder used, and hence to the presumed amount of lipase present. After this time it becomes more

irregular, especially in No. 3, and it remains to account for this variation in the latter stages of the action. As it was suggested that this might be due to the fact that the chloroform did not entirely prevent the development of organisms, another similar test was made in which the whole apparatus was maintained completely sterile. Under these circumstances, the following figures were obtained. In the control flask, the powdered material was added, but the flask containing it was heated in boiling water for half an hour before the oil emulsion was added and the experiment commenced :—

				(1)	(2)	(3)	(4)	(5)
				Control 20 c.c. oil, 0.4 gm. powder previously heated c.c.	20 c.c. oil, 0.1 gm. powder  c.c.	20 c.c. oil, 0.2 gm. powder  c.c.	20 c.c. oil, 0.4 gm. powder  c.c.	20 c.c. oil, 0.8 gm. powder  c.c.
Original acidity	..	..		32.0	22.0	28.0	34.0	50.0
Additional acidity—								
After one	day	..	..	11.0	11.0	21.0	24.0	57.0
„ two	days	..	..	6.0	13.0	24.0	29.0	41.0
„ three	„	..	..	2.0	7.0	10.0	15.0	29.0
„ four	„	..	..	1.5	6.0	7.0	14.5	28.0
„ five	„	..	..	1.0	4.0	5.0	9.0	15.0
„ six	„	..	..	..	2.5	3.0	7.0	8.5
„ seven	„	..	..	..	1.0	3.0	7.0	8.5
„ eight	„	..	..	..	1.0	2.0	6.0	8.0
„ nine	„	..	..	..	..	1.0	5.0	8.0
„ ten	„	..	..	..	..	1.0	4.0	7.0
„ eleven	„	..	..	..	..	..	4.0	4.0
„ twelve	„	..	..	..	..	..	2.0	2.0
„ thirteen	„	..	..	..	..	..	1.0	2.0
„ fourteen	„	..	..	..	..	..	..	2.0
„ fifteen	„	..	..	..	..	..	..	1.0
„ sixteen	„	..	..	..	..	..	..	..
„ seventeen	„	..	..	..	..	..	..	..
TOTAL ADDITIONAL ACIDITY				21.5	45.5	77.0	127.5	221.0

If, as in the last case, we subtract the original acidity in each case, and also the acidity of the control on each day, we have as follows :—

						(2) c.c.	(3) c.c.	(4) c.c.	(5) c.c.
Total acidity—									
After one	day	..	..	..	..	nil	10.0	13.0	46.0
„ two	days	..	..	..	..	7.0	28.0	36.0	81.0
„ three	„	..	..	..	..	12.0	36.0	49.0	108.0
„ four	„	..	..	..	..	16.5	41.5	62.0	134.5
„ five	„	..	..	..	..	19.5	45.5	70.0	148.5
„ six	„	..	..	..	..	22.0	48.5	77.0	157.0
„ seven	„	..	..	..	..	23.0	51.5	84.0	165.5
„ eight	„	..	..	..	..	24.0	53.5	90.0	173.5
„ nine	„	..	..	..	..	24.0	54.5	95.0	181.5
„ ten	„	..	..	..	..	24.0	55.5	99.0	188.5
„ eleven	„	..	..	..	..	24.0	55.5	103.0	192.5
„ fifteen	„	..	..	..	..	24.0	55.5	106.0	199.5

There is thus a very close relationship between the acidity produced and the quantity of seed so far as the action can be due to an enzyme. There are, however, two points which appear from these experiments.

1. The whole of the acidification of the oil cannot be due to enzyme action. In both the experiments quoted there is some decomposition of the oil even where the ferment has been destroyed by heating or when no seed powder was present. In fact it would appear that the mere exposure of safflower oil to the air leads to a certain amount of decomposition, and that this fact may possibly be important in the germination of the seed. This matter demands further investigation.

2. The action of the enzyme is a terminable one, even in the presence of large excess of oil. In each case its increase of acidity arises to a maximum, generally on the second day, and then declines very gradually until action ceases after a time which varies according to the quantity of enzyme present. The larger the quantity of enzyme the longer the action lasts. It would appear, in fact, that the lipase enzyme is destroyed while acting.

So far we have dealt with the presence of a lipase fat-splitting enzyme in the germinating safflower seed, and have shown that such a lipase does exist



here as in other oily seeds in course of germination. It remained to see how far the quantity of this enzyme varied during the germination process. In fact it was desired to answer the question whether actual formation of a lipase enzyme took place during the early life stages of the safflower plant.

To do this, good sound seed was allowed to germinate in sterilized sand and the plants removed when the following stages were reached (*vide* page 228):—

- |                             |  |                                  |
|-----------------------------|--|----------------------------------|
| 1. Radicle just protruding. |  | 3. Lateral roots just appearing. |
| 2. Radicle one inch long.   |  | 4. Root system established.      |

In testing the relative amount of lipase a different method was employed from that previously described. The one now used is due to Tanaka<sup>1</sup> and consisted in treating the pressed seed with tenth-normal acetic acid, and drying the washed-out material at a temperature below 40°C. This gave a powder which could be dried and used for the determination of the lipase.

The actual working of the method was as follows: 0.4 gram of the dried powder thus obtained from each stage of germination was mixed with 10 c.c. of oil emulsified, along with 4 c.c. of water, the whole neutralized (to phenolphthalein) with tenth-normal caustic potash, and incubated at 35°C. In each case two such flasks were prepared, in one of which the enzyme had been destroyed by heating to the temperature of boiling water. By this means any acidification of the oil due to causes other than the enzyme can be eliminated, and the figures reported represent the difference between the acidity developed in the presence of active enzyme and of the same powder in which the enzyme had been destroyed. They are as follows:—

					ACIDITY PRODUCED IN OIL EMULSION
					(Cubic centimeters of 10% oil emulsion for 0.4 gram of dried powder)
Resting seed	..	..	..	..	0.1 (completed in 12 days).
Radicle just protruding	..	..	..	..	0.2 (completed in 11 days).
Radicle one inch long	..	..	..	..	5.0 (completed in 16 days).
Lateral roots just protruding	..	..	..	..	10.6 (completed in 22 days).
Root system established	..	..	..	..	8.3 (completed in 22 days).

<sup>1</sup> *Journal of the College of Engineering, Tokyo, Imperial University* (1910), vol. 5, no. 2, p. 25.



This result is exceedingly interesting when taken in conjunction with the changes in composition of the germinating seed already described (pages 228 and 229). The amount of active enzyme present in the resting seed is exceedingly small, and increases very little in the early stages of germination, or until the radicle has protruded from the seed. Then it rapidly increases as the radicle develops, reaching a maximum when the lateral roots (the true feeding rootlets) begin to develop, and then declines as the regular feeding roots are formed. This would indicate that in the earliest stages of germination in these oily seeds the plant is not dependent on the oil in the seed, but utilizes other materials present, and that only later is the oil a vital factor in the growth of the seedling. The nature of the material (zymogen) from which the lipase is formed has not yet been ascertained.

#### V. THE OXIDASE OF GERMINATING SAFFLOWER SEEDS.

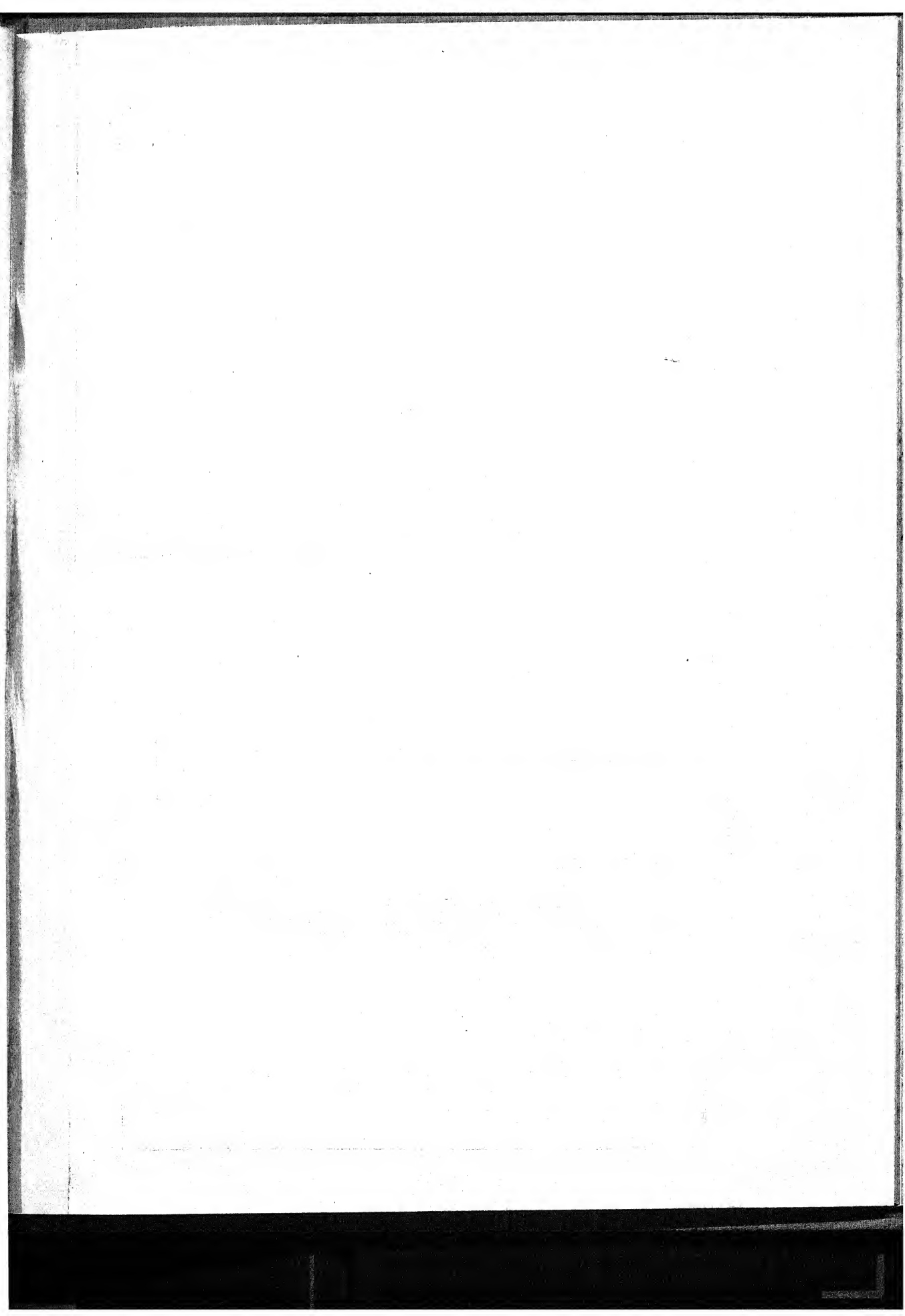
The study of the oxidases known to be present in germinating seeds and of the way in which these vary during the germination has not been undertaken, so far as I can ascertain, in the case of any oily seed such as that of the safflower<sup>1</sup>. Until comparatively recently a really satisfactory method of measuring the oxidase content of plant material was not known. But the perfecting of a method by Bunzel<sup>2</sup> which enables the relative amount of oxidase in a series of materials to be ascertained and which appears exceedingly satisfactory enables the development of oxidase to be followed with ease.

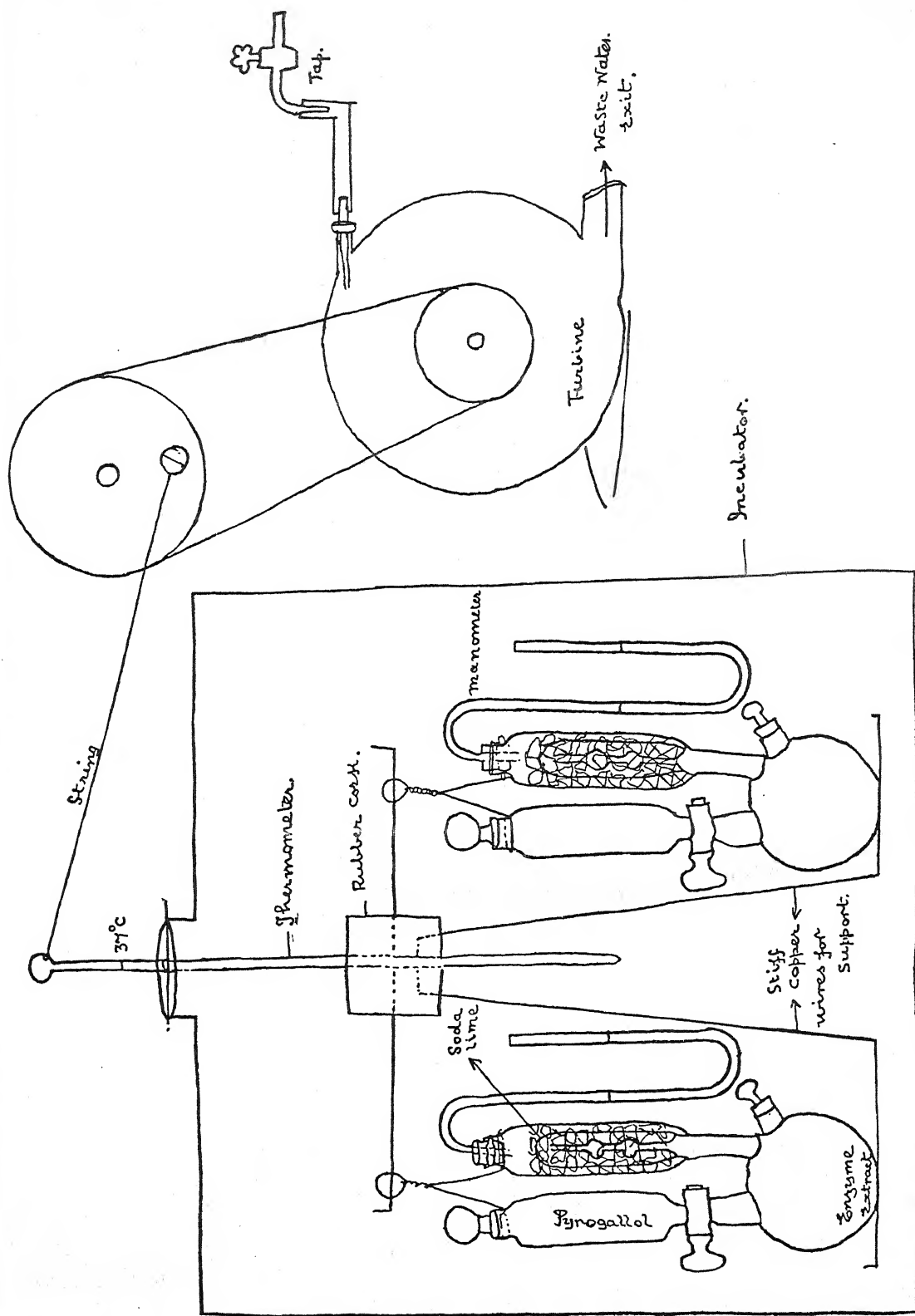
The presence of oxidase in the germinating seed was first shown by crushing the seed at any stage of the process, mixing with water, grinding up the wet mass, and filtering through linen. After washing and squeezing the mass remaining on the cloth, the filtrate was precipitated with three times its volume of alcohol, and, after filtration, the precipitate dissolved in water. The liquid thus obtained always gave a marked blue colour with an alcoholic solution of guaiacum resin which was not deepened by the addition of hydrogen peroxide. After heating the liquid to 90°C. this reaction could no longer be obtained. This reaction, characteristic of oxidases, removed any doubt as to their presence in germinating safflower seed.

The method of Bunzel for determining the amount of oxidase present in any plant material has, somewhat modified as to apparatus, been used throughout these experiments. It depends on the actual measurement of the

<sup>1</sup> Except by Deleano—see Bibliography and also Introduction.

<sup>2</sup> U. S. Dept. Agri. Bureau of Plant Industry Bulletin 238 (1912).





\* The measurement of the oxidase content of plant juices by H. H. Bunzell—Bulletin No. 238, U. S. A. Department of Agriculture as modified by V. A. Tamhane.

Diagram Showing the oxidase apparatus.

amount of oxygen absorbed when the water extract of the ground material is mixed with a solution<sup>1</sup> of pyrogallol and kept at a constant temperature until absorption of oxygen ceases. The important things to secure are (1) a constant temperature, (2) an apparatus in which the carbon dioxide given off can be removed at once, and so the oxygen absorbed measured off directly, (3) a regular shaking of the apparatus during the whole process. These are secured by Bunzel, respectively, by an electrical thermoregulator, by an apparatus of his own devising which allowed the use of a definite quantity of pyrogallol solution, containing a receptacle for the removal of carbon dioxide by caustic potash as soon as formed, and carrying a manometer on which the absorption of oxygen could be directly measured, and by the use of an electrically driven shaking machine external to the thermostat.

Instead of these devices I employed a Hearson's incubator to maintain a constant temperature, a Schroeder CO<sub>2</sub> apparatus to which a manometer was attached as the reaction apparatus, and a small water turbine attached by an eccentric to a piece of glass rod to which the reaction apparatus was slung. The accompanying diagram shows the arrangement<sup>2</sup>.

The whole process for determining the changes in the oxidase content in the germinating seed was as follows :—Good sound safflower seed was steeped in 1 per cent. copper sulphate solution for fifteen to twenty minutes and then sown in sterile sand kept moist with distilled water. When the seedling had grown to the stage desired, it was removed, washed free from adhering sand particles, and pressed lightly in blotting paper. About ten grammes of the seedlings were then weighed out and crushed along with a crystal of thymol as an antiseptic, ten cubic centimeters of water were incorporated with the pulp and the whole mass allowed to stand for half an hour. It was then pressed through cloth, and 4 c.c. of the extract was placed in the apparatus, with 8 c.c. of 1 per cent. pyrogallol solution and 4 c.c. of 2½-normal caustic potash in their proper places. The manometer was then attached and the whole apparatus kept in the thermostat until it had reached a constant temperature of 37°C. with the stopper open to the air. When the temperature was constant, generally after about two hours, the pyrogallol solution was run into the extract, the stopper to the air closed, and the apparatus kept shaken with 25 to 30 complete oscillations per minute.

In about two hours the manometer began usually to show a fall in the pressure, a reading was made and fresh readings were taken at intervals as

<sup>1</sup> Palladin. *Ber. Deutsch. Bot. Gesells.*, vol. 24 (1906), p. 97.

<sup>2</sup> The diagram is from Vol 2, Part XIII, pp. 195-208 of the *Journal of the Indian Institute of Science, Bangalore*, where the apparatus was used after it was devised.

shown in the following tables, the whole test usually lasting about twenty-four hours. The results obtained were found to be constant in different tests with the same material, and closely proportionate to the amount of oxidase containing material employed. The constancy obtained is shown by the following figures :—

*Experiment I.* 2 c.c. oxidase extract taken in each of two cases (1) and (2). Experiment begun at 9 a.m. Constant temperature reached at 11 a.m. and pyrogallol added. Manometer readings (differences) in centimeters were obtained as follows :—

			(1)	(2)
			2 c.c. extract	2 c.c. extract
			cm.	cm.
2 p. m.	..	..	0.2	0.2
4 "	..	..	0.5	0.6
6 "	..	..	1.0	1.2
11 "	..	..	1.0	1.2
8 a. m.	..	..	1.1	1.2

*Experiment II.* Details similar to the last, 4 c.c. extract however being used. Pyrogallol added at 11-30 a.m.

			(1)	(2)
			4 c.c. extract	4 c.c. extract
			cm.	cm.
3 p. m.	..	..	0.25	0.32
6 "	..	..	0.60	0.65
9 "	..	..	1.30	1.52
12 Midnight	..	..	1.92	2.10
3-30 a. m.	..	..	2.12	2.50]
6-30 "	..	..	2.23	2.60
30 "	..	..	2.24	2.60

*Experiment III.* 2 c.c. oxidase extract taken in (1), 4 c.c. oxidase extract taken in (2). Experiment begun at 9-30 a.m. Constant temperature reached at 11 a.m. and pyrogallol added. Manometer readings (differences) in centimeters were obtained as follows :—

		(1)	(2)
		2 c.c. extract	4 c.c. extract
		cm.	cm.
2 p.m.	..	0.1	0.2
4 "	..	0.2	0.5
6 "	..	0.2	0.7
9 "	..	0.3	0.7
10-30 p.m.	..	0.3	0.8
8 a. m.	..	0.4	0.8

*Experiment IV.* Details and times similar to the last.

		(1)	(2)
		2 c.c. extract	4 c.c. extract
		cm.	cm.
1-30 p. m.	..	0.15	0.25
3-30 "	..	0.20	0.50
5-30 "	..	0.35	0.65
8-45 "	..	0.40	0.70
11-30 "	..	0.40	0.70
7-0 a. m.	..	0.40	0.70

*Experiment V.* Details similar to the last, except that in (2) four times the amount of oxidase extract was used than that employed in (1). Pyrogallol added at 1 p.m.

		(1)	(2)
		2 c.c. extract	8 c.c. extract
		cm.	cm.
5-30 p. m.	..	0.10	0.44
8-30 "	..	0.41	1.36
11-30 "	..	0.53	1.80
2-30 a. m.	..	0.65	2.30
5-30 "	..	0.70	2.71
8-30 "	..	0.71	2.71



As the method appeared to give constant results, and as the readings obtained were proportionate to the amount of extract taken, the method was applied to the determination of the relative amounts of oxidase at different stages of germination. As the action seemed to be complete after eighteen to twenty-four hours the reading after this time was taken in each case.

I. In the first series the germination was carried out at the room temperature of 27 to 30°C.

Condition of seed and seedling	MANOMETER READING (DIFFERENCES) AFTER 18 TO 27 HOURS	
	a	b (duplicate)
	cm.	cm.
Resting seed .. .. .	nil	nil
6 hours' germination .. .. .	nil	nil
12 do. do. (radicle just protruding)	0.30	0.35
18 do. do. .. .. .	0.75	0.83
24 do. do. .. .. .	0.90	0.85
36 do. do. .. .. .	1.05	0.99
48 do. do. .. .. .	2.81	2.42
72 do. do. .. .. .	2.60	2.45
96 do. do. .. .. .	1.19	1.25

In this case the stage of germination reached may be considered to be as follows :—

- (a) After 12 hours the radicle is just protruding.
- (b) After 48 hours the radicle is well developed but without lateral roots.
- (c) After 72 hours the lateral roots are just commencing to spread.
- (d) After 96 hours the root system is established.

This being so, the increase in the proportion of oxidase closely corresponds with the increase previously shown to occur with the lipase. The quantity is negligible or very small until the growth of the radicle is considerable ; it reaches a maximum just before the lateral roots begin to form and thereafter rapidly diminishes as the capacity for absorbing outside food is developed.

II. A second partial experiment at the same temperature gave similar results.

Condition of seed and seedling	MANOMETER READING WHEN CONSTANT (AFTER 18 TO 27 HOURS)	
	a	b (duplicate)
	cm.	cm.
Resting seed .. .. .	nil	0.20
6 hours' germination .. .. .	0.20	0.20
12 do. do. .. .. .	0.60	0.70
24 do. do. .. .. .	0.82	0.89

The figures are closely similar to those in the previous experiment except that the development of oxidase commences a little earlier.

III. A third series of experiments was now undertaken in which the germination was carried out at a higher and more constant temperature, namely, at 37°C. in a thermostat. In all other respects the method was identical with that just described.

Condition of seed and seedling	MANOMETER READING (DIFFERENCES WHEN CONSTANT AFTER 18 TO 27 HOURS)	
	a	b (duplicate)
	cm.	cm.
a. 6 hours' germination .. .. .	nil	0.15
12 do. do. .. .. .	0.61	0.72
18 do. do. .. .. .	0.83	0.94
24 do. do. .. .. .	1.20	1.11
36 do. do. .. .. .	2.30	2.53
48 do. do. .. .. .	2.81	2.95
72 do. do. .. .. .	2.83	2.42
b. 6 hours' germination .. .. .	0.20	0.10
12 do. do. .. .. .	0.60	0.57
24 do. do. .. .. .	1.15	1.28
72 do. do. .. .. .	2.56	2.39

When germination was carried out at 37°C. the root system was established after 72 hours, and if the experiment was continued for 96 hours the seedlings begin to show signs of decay. From general growth the seedlings were about six hours ahead of those at the room temperature of 27° to 30°C.

The general conclusions are however the same in the two cases. There is no oxidase in the resting seed, but it appears in minute quantity as soon as the radicle is formed. The proportion increases rapidly during the growth of the radicle, reaching its maximum when the lateral roots are commencing to form. After this it gradually declines, and it would appear as if it was not necessary in anything like such large proportion during the normal later life of the plant. After germination is over its amount probably varies very much with the amount of plant food supplied, and this introduces another factor demanding separate investigation.

#### VI. SUMMARY AND CONCLUSIONS.

The conclusions which are reached as a result of the experiments described may be summarized as follows:—

(1) The safflower seed contains reserve materials chiefly in the form of oil and proteid matter. In the resting seed there are no starch, no glucosides, and no tannin, and a small proportion of non-reducing sugars (0.37 per cent.) capable of inversion. This does not seem to be cane sugar, but its identification has not yet been definitely made.

(2) During germination the composition, so far as the relative amount of oil, proteids and nitrogen-free extract is concerned, changes little until the radicle protrudes from the seed. Changes then rapidly occur. The oil disappears and a corresponding increase in the nitrogen-free extract takes place. This increase in the latter is not due to the formation of starch, which appears to be absent throughout. On the other hand sugars appear, non-reducing sugars gradually increasing as the germination becomes complete. The reducing sugars appear to rise to a definite proportion and then vary little. The proteid matter is not very largely solubilized until after the radicle has grown to a considerable extent. When the lateral roots are about to form and after this stage it is rapidly converted into a soluble form.

(3) The lipase occurs, as was found by Green in castor oil seed, very largely in the form of a zymogen, which is converted into the active condition during the germination itself. The amount of active enzyme in the resting seed is in fact exceedingly small and increases very little in the early stages of germination or until the radicle has protruded from the seed. Then it rapidly increases as the radicle develops, reaching a maximum when the lateral roots

begin to appear and then declines as the regular feeding roots are formed. It would appear as if the plant is not dependent on the oil in the earliest stages of germination, but first utilizes other materials present. Only later is the oil a vital factor in the growth of the seedling.

The experiments cited lead also to the conclusion that all the acidification of the oil during germination is not due to enzyme action. What the other cause may be has not been so far ascertained. Further, the action of the lipase seems to be a terminable one and hence the effect produced, as a certain quantity is limited. Whether the enzyme is destroyed during its action, or its activity is limited by the products which it forms, is not yet clear.

(4) The oxidase either occurs entirely in the form of a zymogen or it is a direct product of the cell metabolism in the early stages of germination. It is not found in the resting seed, but appears in minute quantity as soon as the radicle is formed. The proportion increases rapidly during the growth of the radicle, reaching its maximum when the lateral roots are commencing to form. After this it gradually declines and it would appear as if it was not necessary in anything like such a large proportion during the later life of the plant. At any rate its proportion declines in the absence of a supply of outside plant food. It is interesting to note that during germination the rise in the amount of oxidase follows a little the decomposition of the oil, and the rise in the amount of nitrogen-free extract in the germinated seedling.

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# NOTE ON THE PERMANENT MANURIAL PLOTS, COIMBATORE.

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THE permanent manurial plots at the Central Farm, Coimbatore, were laid out in September, 1909, and have, therefore, now been under observation for a period of nearly thirteen years. During this time thirty-six crops have been grown and the results obtained seem of sufficient interest to place on record.

The plots are 4 cents in area and ten in number. Eight of these receive mineral manures, *i.e.*, nitrogen, phosphoric acid or potash, alone or in combination, while the last two have been treated with cattle manure, the general scheme being as follows :—

<i>Plot</i>		<i>Manure</i>
1	..	.. No manure.
2	..	.. Nitrogen.
3	..	.. Nitrogen plus potash.
4	..	.. Nitrogen plus phosphate.
5	..	.. Nitrogen plus potash plus phosphate.
6	..	.. Potash plus phosphate.
7	..	.. Potash.
8	..	.. Phosphate.
9	..	.. Cattle manure. (Continuous.)
10	..	.. Cattle manure. (Application stopped in 1916.)

The manures in use have been—

Ammonium sulphate 1 cwt. per acre for nitrogen.

Potassium sulphate 1 cwt. per acre for potash.

Superphosphate 3 cwt. per acre for phosphate.

Cattle manure 5 tons per acre for plots IX and X.



Analyses of the plots at the time they were laid down do not seem to have been made, but samples were collected in 1916 from which the following figures are taken. The experiment had then been in progress for seven years.

*Analyses, Plots I and V. 1916.*

	Plot I. No manure	Plot V. N+K+P. Complete mineral manure
Moisture .. .. .	2.000	2.230
Loss on ignition .. .. .	2.390	2.130
Insoluble mineral matter .. .. .	89.290	88.020
Ferric oxide ( $\text{Fe}_2\text{O}_3$ ) .. .. .	4.750	5.990
Alumina ( $\text{Al}_2\text{O}_3$ ) .. .. .		
Lime ( $\text{CaO}$ ) .. .. .	0.850	0.750
Magnesia ( $\text{MgO}$ ) .. .. .	0.490	0.560
Potash (total) ( $\text{K}_2\text{O}$ ) .. .. .	0.230	0.290
Soda ( $\text{Na}_2\text{O}$ ) .. .. .	0.007	0.007
Phosphoric acid (total) ( $\text{P}_2\text{O}_5$ ) .. .. .	0.031	0.059
Sulphuric acid ( $\text{SO}_3$ ) .. .. .	0.021	0.027
Carbonic acid ( $\text{CO}_2$ ) .. .. .	0.625	0.388
Nitrogen .. .. .	0.0290	0.030
Available phosphoric acid ( $\text{P}_2\text{O}_5$ ) .. .. .	0.0076	0.043
Available potash .. .. .	0.0170	0.020

The soil of the unmanured plot is thus typical of many in the Madras Presidency exhibiting a low content of organic matter and a marked deficiency of nitrogen and available phosphoric acid, while potash in an available form is present in considerable quantity. A feature of the figures in the fully manured plot is the marked accumulation of available phosphoric acid, the amount of nitrogen remaining in the soil being, however, almost unchanged.

The cultivation of the plots has been intensive in order to emphasize the differential action of the manures. Irrigation was given as required to all crops and the plots dug over by hand in the intervening periods. In this way it has been possible to take off three crops a year or thirty-six in all since the experiment started.

The plots were originally laid down simply to form a series of demonstration plots for students of the College. With this object in view it was, of course, not necessary to maintain such complete records as would have been done if the plots had definitely been started as a series of permanent manurial experiments for scientific study. Consequently in the early years analyses of the straws were not made so that it is now impossible to compute the total amount of plant food removed by the crops from the various plots. The plots were

also not completely fenced in and the cultivation sheets bear record of occasional damage to particular plots by stray cattle. At a later period, again, the plots were undoubtedly affected by the proximity of a row of rain trees (*Pithecolobium Saman*), though as the row ran across the end of the plots one may perhaps assume that all suffered more or less equally. It was, however, soon realized that the plots were yielding results of considerable interest and the original scheme was enlarged in order that complete records might be available.

In Table I, the yields of the various crops have been collected, the figures representing pounds per acre.

The results, however, can be much more clearly visualized in the graphs and diagrams which follow.

In Plate I, fig. 1, the yields have been plotted to show the result of adding nitrogen, phosphoric acid or potash singly, the value of the no-manure plot being taken as 100.

In the earlier years, as might be expected, the curves run closely together though the nitrogen plot is generally slightly ahead. A definite change occurs, however, about crop 18 and from that time onwards the phosphate plot is always in front, the difference becoming rapidly more marked as the experiment continues. This effect is perhaps more clearly shown in Plate I, fig. 2, where the yields of the complete manure plot and of three plots receiving each two manurial ingredients are plotted against the no-manure plot, the latter again being taken as 100. Here the plot omitting phosphate is from the time of crop 18 definitely below the others. This effect becomes strikingly emphasized by crop 28, from which time it is obvious phosphate has definitely become a limiting factor. The plots containing phosphate are not widely different though that omitting nitrogen (*i.e.*, K plus P) remains below the "complete" and the "nitrogen plus phosphate" plots.

In Plate II, fig. 1 the "no-manure" plot at 100 is compared against "nitrogen only" and "nitrogen plus phosphoric acid." Up to crop 13 the addition of nitrogen alone produces much the same result as nitrogen plus phosphate. By this time, however, the available phosphate in the non-phosphate plots is becoming exhausted and the addition of nitrogen alone henceforth has less effect, the yield now being limited by the phosphate content. The same result is shewn in a different form in Plate II, fig. 2 which illustrates the effect of adding phosphate to nitrogen plots. From crop 13 onwards the effect of the added phosphate is more and more marked and ultimately becomes very striking indeed.

In Plate III, the "complete mineral" plot is compared against the farmyard manure plots, the control "no-manure" being as usual represented by 100. All the curves keep closely together until crop 22 is reached. From that time onwards the application of manure to plot 10 was discontinued and the curve for that plot from that time, therefore, shows the residual effect of the cattle manure previously applied. It is clear this has persisted through all eight crops which have followed. No significant difference is perceptible between the yields of the "complete mineral" and "continuous cattle manure" plots.

Plates I—III, referred to above, include the results for all the crops grown which embrace some seven or eight varieties. It is, however, of greater interest perhaps to examine the effect on individual crops. Two only have been repeated a sufficiently large number of times to yield figures of value, these being *cholam* (*Sorghum vulgare*) grown ten times and *ragi* (*Eleusine coracana*) of which there have been seven successful crops. The average yields of grain and straw obtained for these crops on the various plots are shown in Plates IV—VI.

Fig. 1 on Plate IV shows the average results for all the *cholam* crops grown, while fig. 2 covers the period from crop 24, i.e., after phosphate starvation had become established in the non-phosphate plots. Plate V shows the straw values for the latter period, while in Plate VI are illustrated the results obtained with *ragi*. The relative yields compared to "no-manure" at 100 are also set out in Tables II and III below which, therefore, show the percentage increase due to the manure employed. Table IV gives similar figures for wheat but only four crops of the latter have been grown.

TABLE II.  
*Relative yields of cholam. No manure=100.*

Plot	Average of all crops (Plate IV, fig. 1)	Average of crops from 24 onwards (Plate IV, fig. 2 and Plate V)	
		Grain	Straw
No manure .. ..	100	100	100
N .. ..	124	112	126
K .. ..	125	123	143
P .. ..	168	232	163
N plus P .. ..	182	292	191
N plus K plus P .. ..	222	275	192
N plus K .. ..	139	111	140
K plus P .. ..	203	253	167
Cattle manure .. ..	235	295	192

TABLE III.

*Relative yields of ragi. Plate VI. No manure=100.*

Plot				Average of crops from 28 onwards	
				Grain	Straw
No manure	..	..	..	100	100
N	..	..	..	170	165
K	..	..	..	300	200
P	..	..	..	529	286
N plus P	..	..	..	812	449
N plus K plus P	..	..	..	903	548
N plus K	..	..	..	226	181
K plus P	..	..	..	675	414
Cattle manure	..	..	..	750	426

TABLE IV.

*Relative yields of wheat. No manure=100.*

Plot				Average of all crops	
				Grain	Straw
No manure	..	..	..	100	100
N	..	..	..	157	135
K	..	..	..	138	126
P	..	..	..	161	137
N plus P	..	..	..	224	195
N plus K plus P	..	..	..	246	230
N plus K	..	..	..	158	120
K plus P	..	..	..	194	183
Cattle manure	..	..	..	171	191

It is quite clear from Plates IV—VI and from Tables II, III, and IV, that phosphate has become a controlling factor in these plots. There is always a considerable response to nitrogen but except in the case of wheat this is always very much less than the increase due to phosphate, and in those plots where no phosphate is applied the nitrogen increase is evidently limited by the deficiency of phosphoric acid. For it will be seen on comparing figs. 1 and 2 of Plate VI that the increase due to nitrogen alone or nitrogen plus potash is very much less in the later crops than in those grown before the phosphate supply became so limited.

The action of potash is less clear. In view of the large amount of "available potash" shown by analysis to be present in the soils of all the plots, one would have expected little result to follow its application. This, however,

has not invariably proved to be the case. The most marked action of potash has been exhibited with *ragi* where its application has resulted in a large increase both of grain and straw (Table III). In the case of *chulam* it has had much less effect, and with wheat also the increase due to potash in the presence of other manures has not been marked. Though the amount of "available" potash in the soil is considerable, this can evidently not always be taken up so easily by the crop as the very soluble potassium sulphate applied to the potash plots.

So far the *yield* of crop has alone been taken into consideration. It is of interest, however, to examine the variations in the composition of the crops and the relative proportion of grain and straw.

### Composition of crop.

TABLE V.

*Variation in nitrogen, phosphoric acid and potash content of cholam grain.*  
Average figures for all crops grown.

Plot	PERCENTAGE COMPOSITION OF GRAIN		
	Nitrogen	Potash (K <sub>2</sub> O)	Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )
No manure .. ..	1.843	0.399	0.549
Nitrogen .. ..	1.821	0.401	0.552
Nitrogen plus potash .. ..	1.755	0.349	0.557
Nitrogen plus phosphate .. ..	1.788	0.439	0.776
Nitrogen plus phosphate plus potash .. ..	1.754	0.456	0.817
Phosphate .. ..	1.858	0.449	0.794
Phosphate plus potash .. ..	1.788	0.464	0.822
Potash .. ..	1.798	0.398	0.618
Cattle manure .. ..	1.810	0.433	0.624

NITROGEN ..	{	Average nitrogen plots ..	1.780	Average phosphate plots ..	1.797
		Average non-nitrogen plots ..	1.827	Average non-phosphate plots ..	1.804
POTASH ..	{	Average potash plots ..	0.433	Average phosphate plots ..	0.452
		Average non-potash plots ..	0.428	Average non-phosphate plots ..	0.399
PHOSPHATE ..	{	Average phosphate plots ..	0.801		
		Average non-phosphate plots ..	0.568		

From an examination of these figures it is clear that so far as nitrogen is concerned there is no significant variation in the composition of the grain between any of the plots. The application of nitrogen has improved the crop yield but not increased the store of nitrogen in the grain, nor has the latter been influenced by the amount of phosphate.

In the case of potash also, the percentage of this substance in the grain has not been increased by adding further potash to the soil, the average results of the potash and non-potash plots being practically identical. If, however, the comparison be made, instead, between those plots receiving phosphate and those without, it will be seen that the addition of phosphate has enabled the grain to take up more potash, there being an average difference of 10 per cent. between the potash content in the two series. As pointed out above, no such difference due to phosphate is evident in the nitrogen figures.

It is, however, in the phosphate content of the crop that the most striking variation is seen, there being a difference of 41 per cent. in the results, comparing the non-phosphate plots against those receiving phosphate. If the later crops only are considered, that is to say those grown after the phosphoric acid deficiency in the non-phosphate plots had become marked, the variation is naturally even more noticeable as will be seen from the table below.

TABLE VI.

*Phosphoric acid content of cholam. Average of crops from Number 24 onwards.*

Plot					Percentage of phosphoric acid	
					Grain	Straw
No manure	..	..	..	..	0.481	0.082
Nitrogen	..	..	..	..	0.490	0.073
Nitrogen plus potash	..	..	..	..	0.491	0.071
Potash ..	..	..	..	..	0.584	0.094
Average non-phosphate plots .. ..					0.511	0.080
Phosphate	..	..	..	..	0.795	0.159
Phosphate plus nitrogen	..	..	..	..	0.810	0.189
Phosphate plus nitrogen plus potash	..	..	..	..	0.808	0.175
Phosphate plus potash	..	..	..	..	0.871	0.184
Average, phosphate plots .. ..					0.821	0.177
Per cent. increase over non-phosphate plots ..					60.0	121.2



The results tabulated in Tables V and VI are also shewn graphically in Plate VII. This indicates quite clearly how after crop 24, as phosphate exhaustion became more and more marked, the curves for the phosphate and non-phosphate plots respectively tend to diverge into two separate groups, the phosphate content of the crops from the non-phosphate plots gradually diminishing.

The importance, therefore, of an adequate supply of phosphate is twofold, for not only does any deficiency reduce the yield, but it also seriously diminishes the food value of the crop both in the grain and in the straw. This is made clear by the figures quoted in Table VII below which shows the amount of phosphoric acid removed per acre from the various plots in the grain. Since this amount depends both on the yield and on the phosphoric acid content of the grain, both of which are diminished in phosphate starvation, the contrast between the phosphate and the non-phosphate plots is very striking.

TABLE VII.

*Average weight of phosphoric acid removed per acre in the grain.  
Crop cholam.*

Plot						Phosphoric acid removed per acre in grain
						lb.
No manure	..	..	..	..	..	4.2
Nitrogen	..	..	..	..	..	5.4
Nitrogen plus potash	..	..	..	..	..	5.2
Nitrogen plus phosphate	..	..	..	..	..	13.8
Nitrogen plus potash plus phosphate	..	..	..	..	..	15.3
Potash plus phosphate	..	..	..	..	..	13.4
Potash	..	..	..	..	..	6.05
Phosphate	..	..	..	..	..	10.6

*Average*

lb.

Phosphate plots .. .. 13.25

Non-phosphate plots .. .. 5.22

Considering the large areas in the Madras Presidency in which such a deficiency of phosphate actually exists, it is obvious that we have here a very serious source of loss and the importance of conserving all possible supplies of phosphatic manures becomes increasingly urgent.

**Proportion of straw and grain.**

In Table VIII below figures are shewn indicating the proportion of grain to straw in the various plots, the crop under consideration again being *chulam*.

TABLE VIII.

*Proportion of grain to straw. Crop chulam.*

Plot	Ratio grain and straw	
	Straw=100	
	Grain	
No manure .. .. .	13.7	
Nitrogen .. .. .	14.6	
Nitrogen plus potash .. .. .	13.3	
Nitrogen plus phosphate .. .. .	18.2	
Nitrogen plus potash plus phosphate .. .. .	20.9	
Potash plus phosphate .. .. .	20.1	
Phosphate .. .. .	19.0	
Potash .. .. .	14.5	
Cattle manure .. .. .	23.7	

*Average*

Non-phosphate plots ..	14.27	} Increase 37.2 per cent.
Phosphate plots ..	19.55	
Cattle manure ..	23.7	

The results are also shewn graphically in Plate VIII, fig 2. Here again the phosphate has a marked action, increasing the proportion of grain to straw, whereas the addition of nitrogen has had but little effect. This is not altogether in agreement with results found in Europe where phosphate has usually little influence on the grain to straw ratio. On the other hand, Hellriegel, using sand cultures, found the proportion of grain to rise as phosphate was added. It is probable, therefore, that this effect is only exhibited when the phosphate supply reaches a very low figure.

**Summary.**

(1) In the early years of the experiment the plots responded to both nitrogen and phosphoric acid.

(2) The phosphate has been more rapidly exhausted than the nitrogen and has now become a limiting factor so that the addition of nitrogen alone produces but a small increase of crop, whereas the effect of phosphate becomes more marked each year.

(3) Addition of potash has not had any consistent effect in the case of *cholam* or wheat but has materially increased the yield of *ragi* both in grain and straw, though chemical analyses indicated that the soil was already well supplied with available potash.

(4) The percentage of nitrogen or potash in the grain has not been increased by the addition of either or both of these substances to the plots.

(5) The percentage of phosphate in both grain and straw varies with the amount of phosphate available, the average difference in the case of *cholam* between the phosphate and non-phosphate plots being 60 per cent. in the grain and 122 per cent. in the straw. The addition of phosphate has also enabled the grain to take up a further supply of potash, though the difference is not in this case anything like so marked.

(6) The proportion of grain to straw has been but little influenced by the addition of nitrogen or potash but has been raised by the application of phosphate.

I am indebted to Mr. R. Cecil Wood, until recently Superintendent of the Central Farm, who kindly had Plates I—III prepared in his office before leaving India.

AGRICULTURAL COLLEGE,

COIMBATORE,

17th May, 1922.

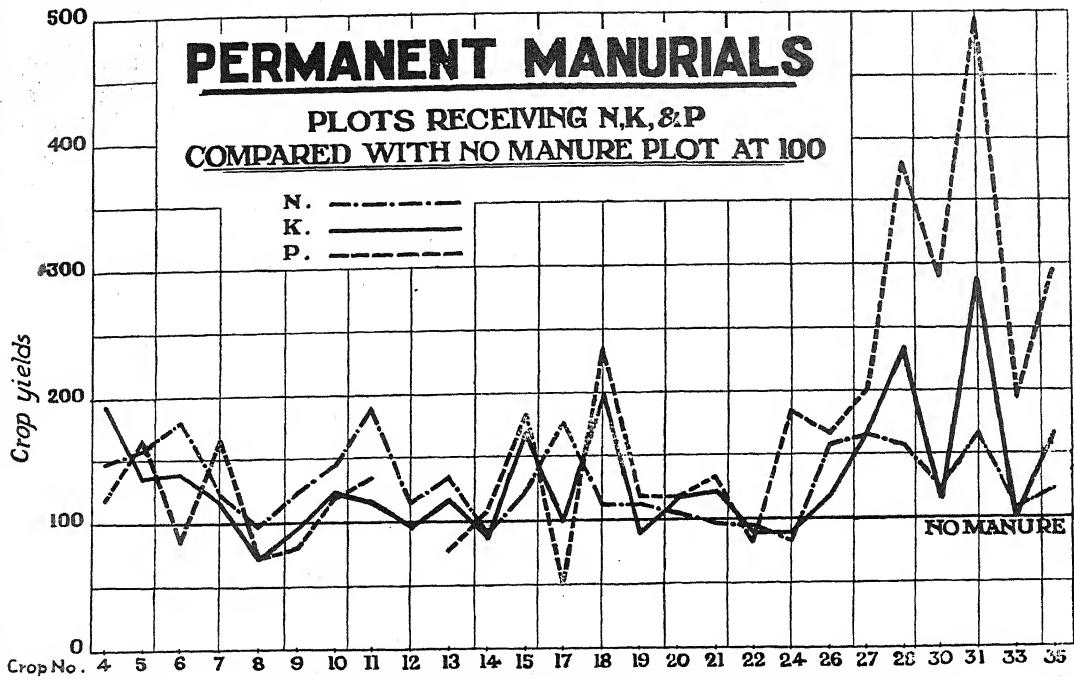


Fig. 1.

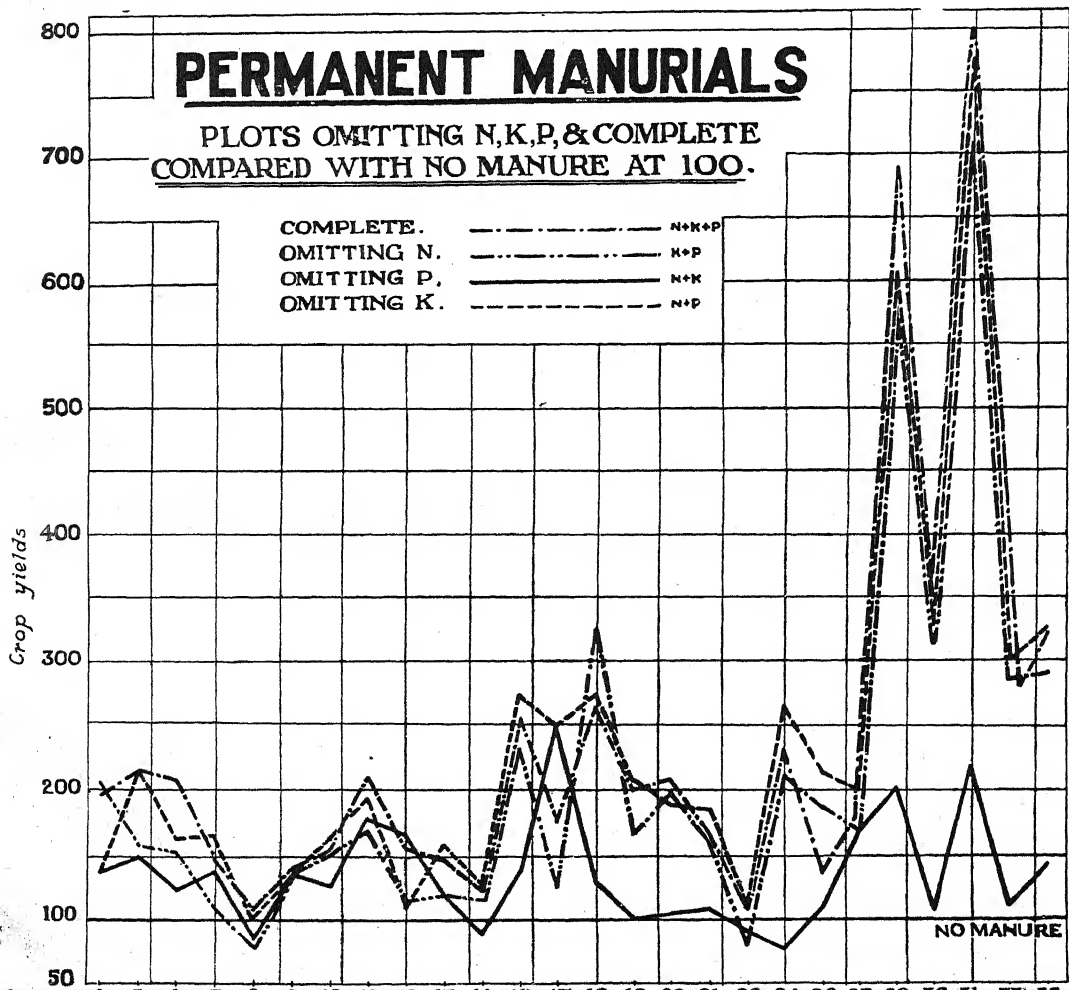


Fig. 2.



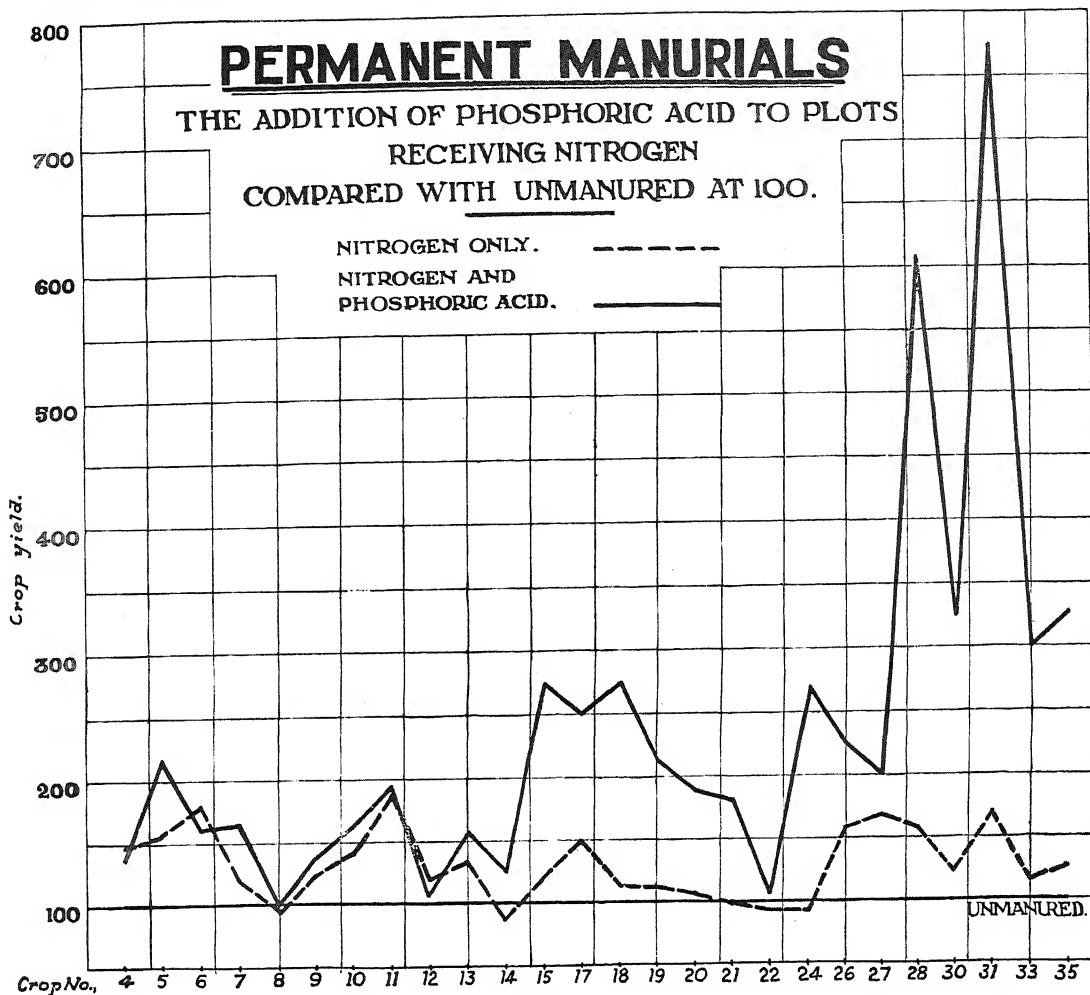


Fig. 1.

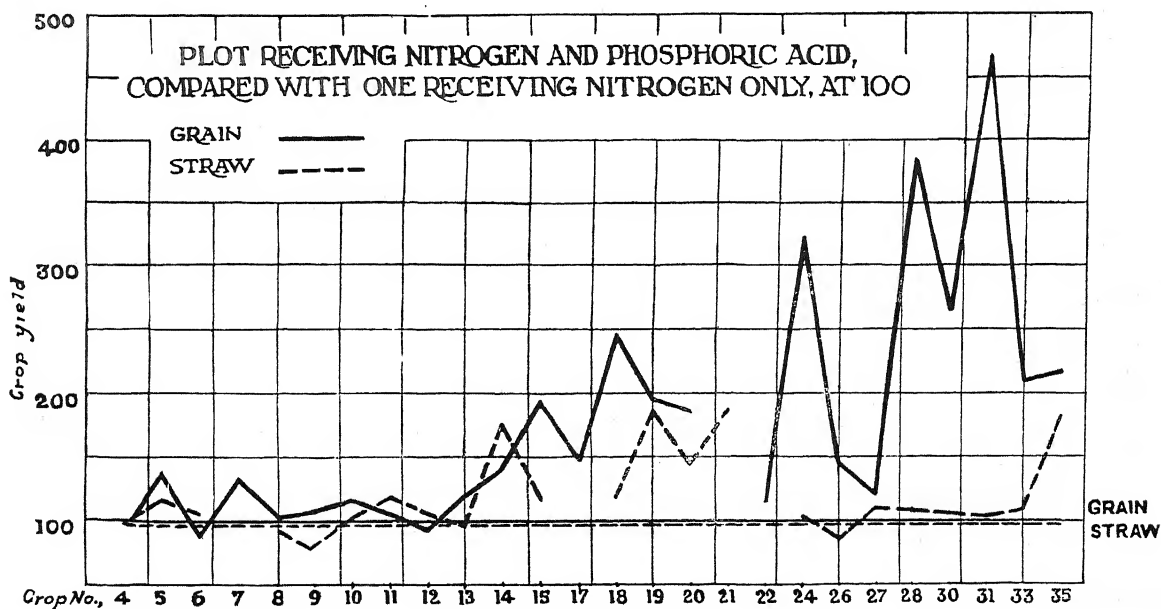
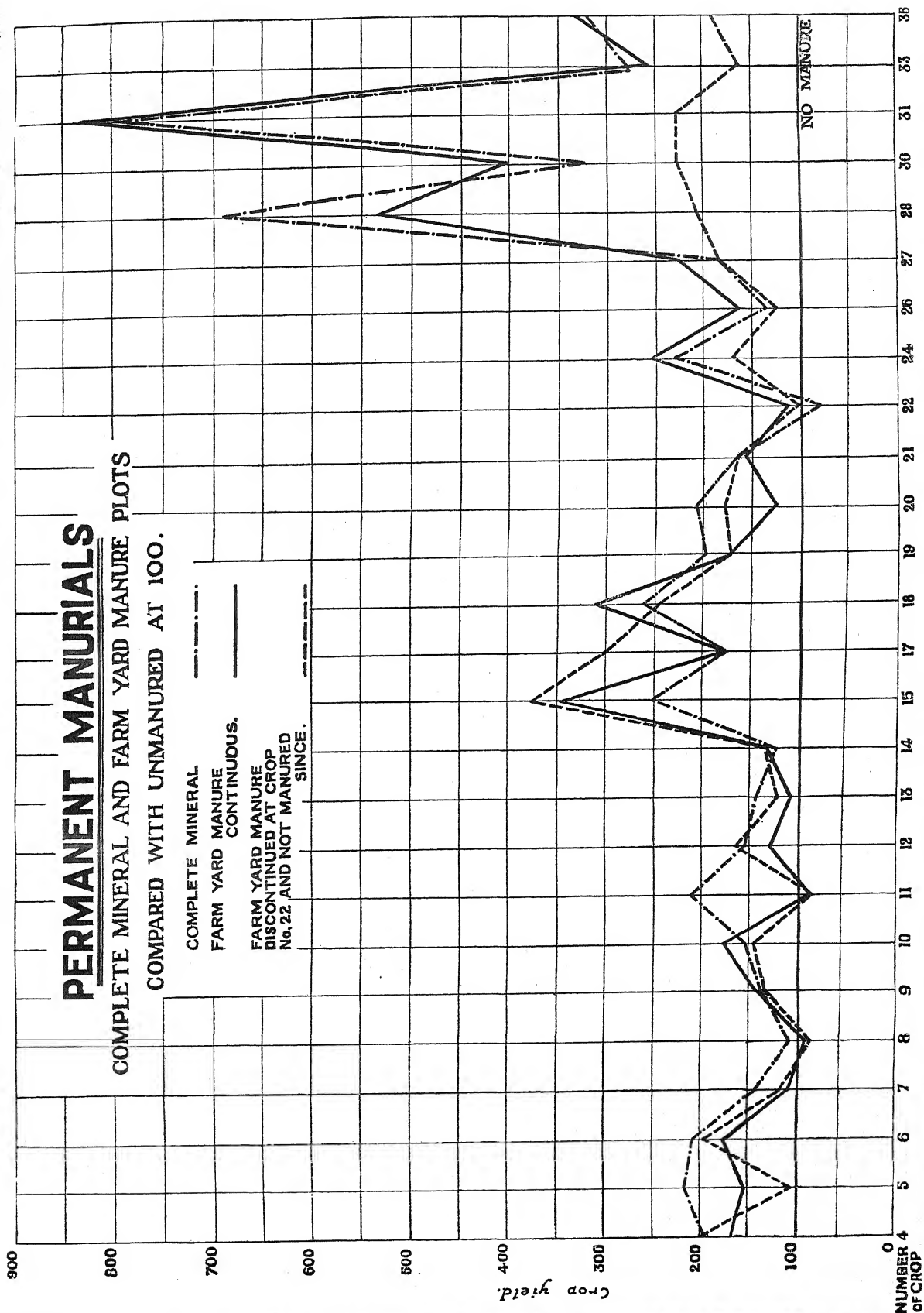


Fig. 2.









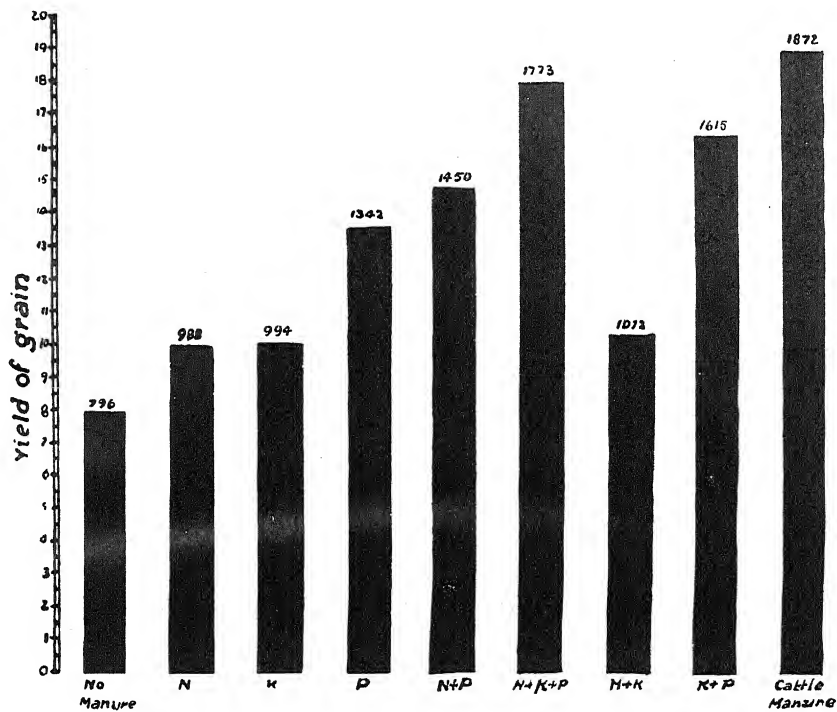


Fig. 1. Relative yields of *cholam* grain. Average crops 6—35.

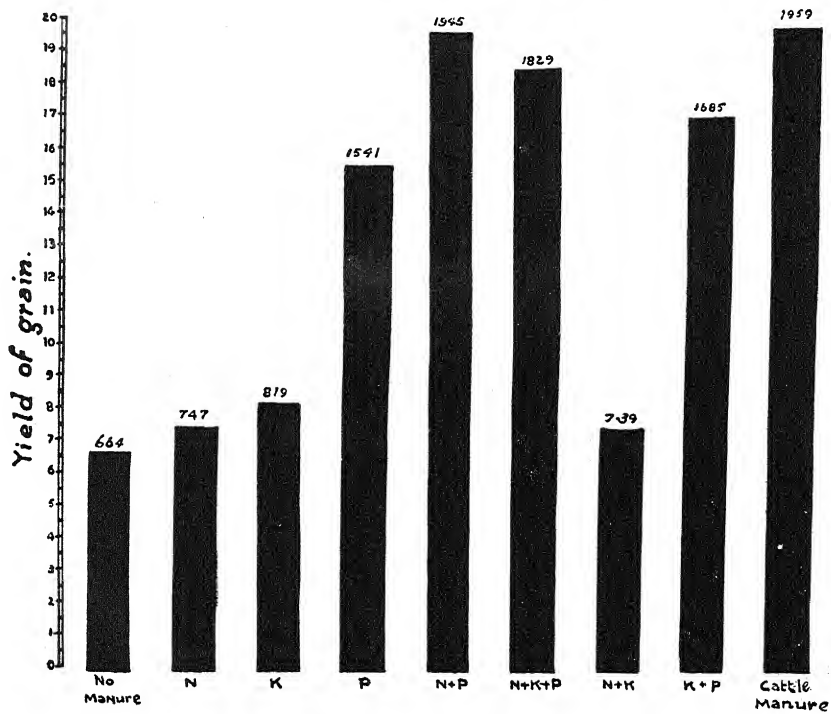
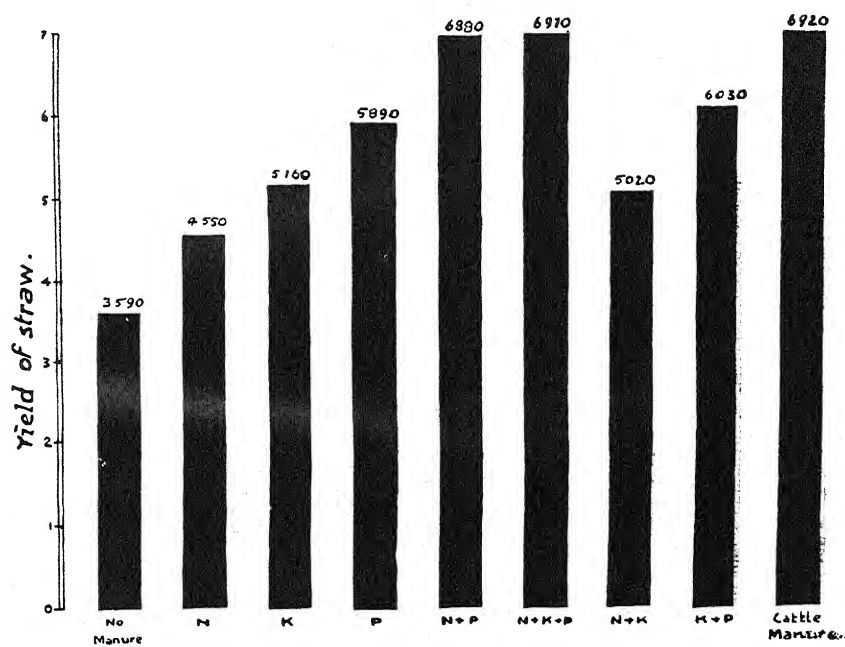


Fig. 2. Relative yields of *cholam* grain. Average crops 24—35





Relative yields of *cholam* straw. Average of crops 24—35.





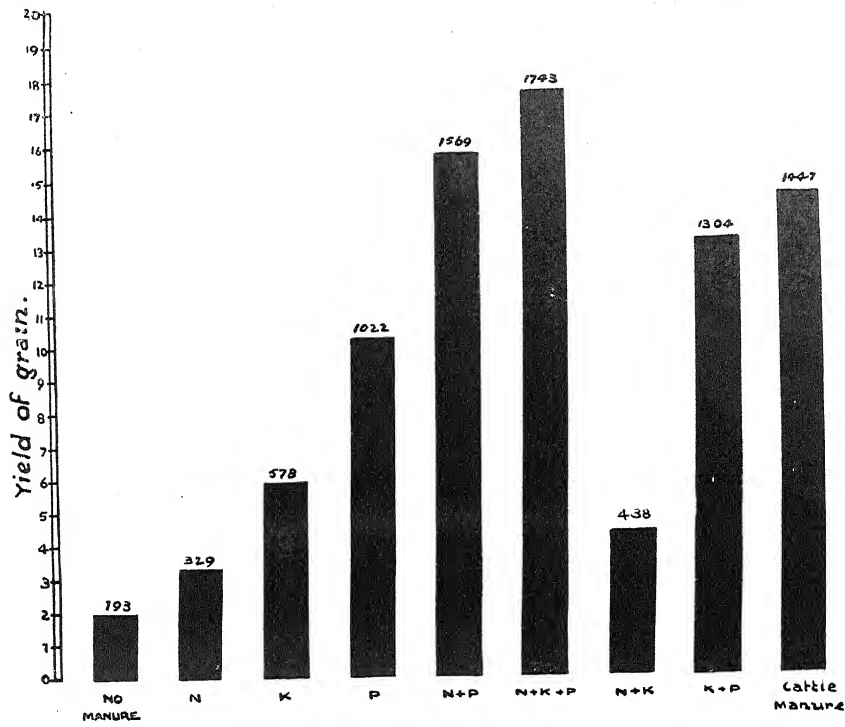


Fig. 1. Relative yields of *ragi* grain. Average of crops 28—36.

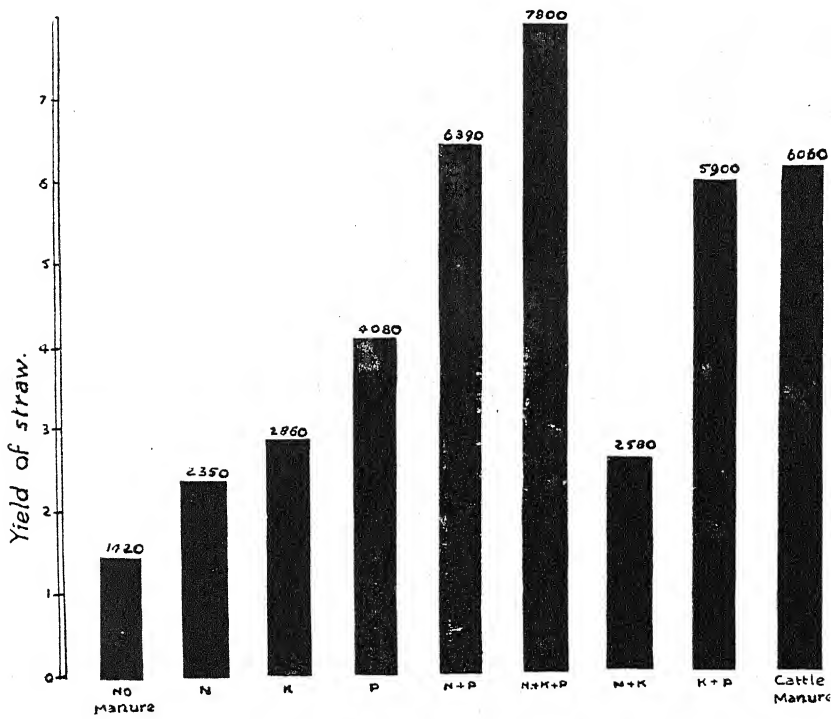
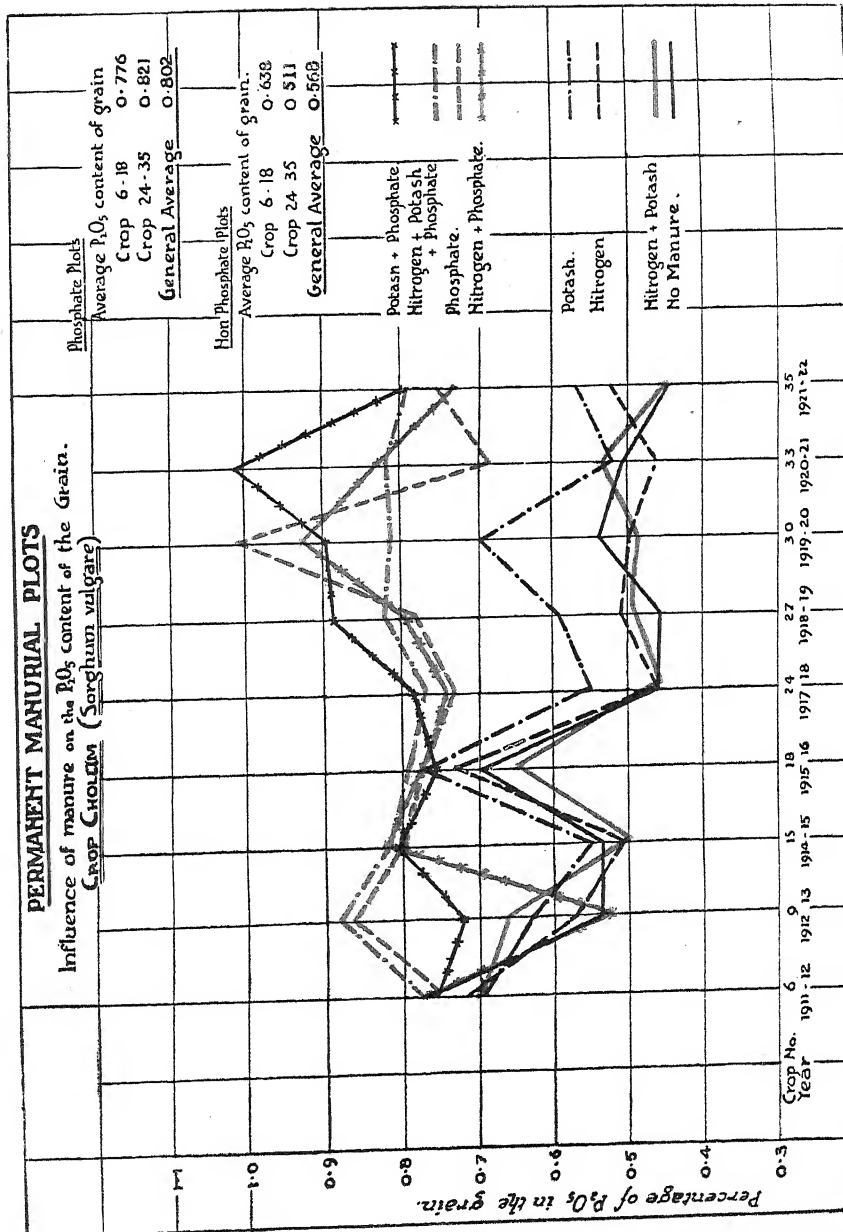


Fig. 2. Relative yields of *ragi* straw. Average of crops 28—35.







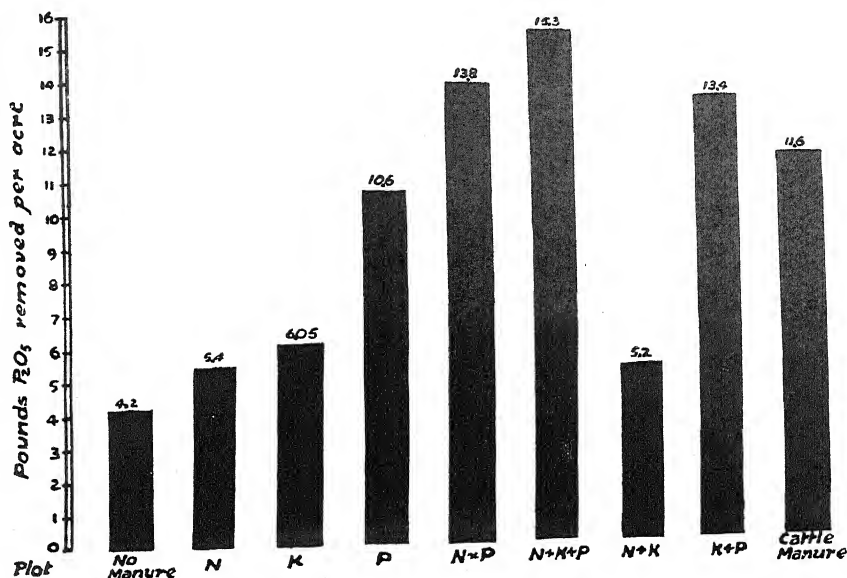


Fig. 1. Weight of  $P_2O_5$  removed per acre from the various plots in *cholam* grain. Average results of all crops.

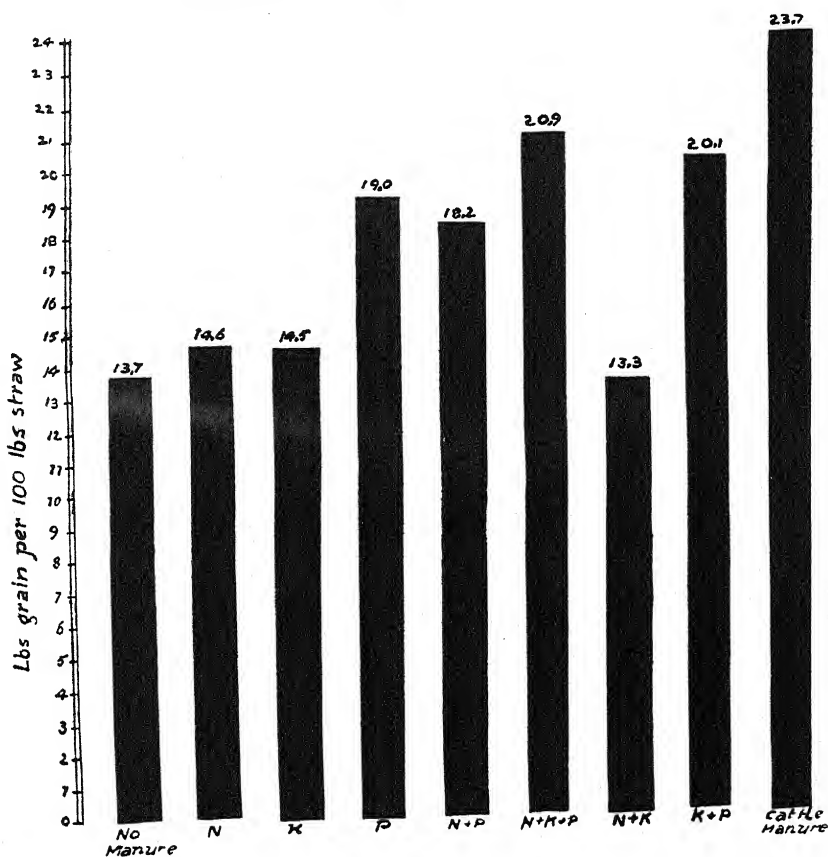


Fig. 2. Proportion of grain to straw. Crop *cholam*. Straw = 100.